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A revision of this paper appeared in the BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA Volume 7, Number 2, pp. 59-62, June 1961. Authors and coauthors of manuscripts intended for publication by the Society should consult this revision carefully before preparing such manuscripts for publication.

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Volume 54 NOVEMBER, 1961 No. 6

The Rearing of *Phlebotomus* Sandflies (Diptera: Psychodidae) I. Technique[1]

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ABSTRACT

The methods and equipment described are used in rearing *Phlebotomus* sandflies, including: rearing vessels; larval food; control of molds; containers for capturing and maintaining adults; releasing cages; feeding cages; aspirator devices for capturing sandflies and loading feeding cages and other containers.

Since early in this century *Phlebotomus* sandflies[2] have been reared by a number of investigators concerned with these insects as annoying pests or in relation to sandfly fever, leishmaniasis or bartonellosis. A considerable variety of methods and types of apparatus have been devised to meet the basic requirements for sandfly rearing, which are: (i) providing the gravid females a moist surface for oviposition, (ii) a moist environment for development of the eggs and immature stages, (iii) suitable food for the larvae, (iv) containers or cages for the emerging adults and their subsequent confinement, feeding and experimental manipulation.

It is not the purpose of this paper to review all the various techniques but rather to give an account of those methods which have proved successful with the various species we have had occasion to rear. The experience of the senior author began in North China in 1924 (Young and Hertig 1926) in connection with studies on kala azar, with the rearing of *Phlebotomus chinensis, P. mongolensis* and *P. squamirostris*, followed by the rearing of *P. vexator* in the United States and of *P. verrucarum, P. noguchii* and *P. peruensis* in Peru, the last three in work on bartonellosis (Hertig 1942).

With the initiation in 1956 of the current study of the leishmaniasis transmission problem in Panama, it was found that our Panamanian species required a number of modifications of the methods which had worked well with several species in three continents. The adaptation of the technique to the peculiarities of our local species was continued by the junior author when she joined the staff of the project (6 months in 1957, January 1959 to the present) and assumed general charge of the rearing program. Part II of this series (Johnson and Hertig 1961) covers in detail the development and behavior in laboratory cultures of our principal man-biting species, together with notes on others of the 24 species, out of the 65 species known to occur in Panama, which we have reared. The taxonomy and distribution of *Phlebotomus* in Panama and elsewhere in the Americas have been the subjects of a series of papers by Fairchild and Hertig, most of which have appeared in the Annals of the Entomological Society of America, 1947 to 1959. These papers also contain incidental information about diurnal shelters of the adults and methods of collecting. It is not the intention, in the present paper, to go into the field aspects of *Phlebotomus*. It may be mentioned, however, that sandflies may be collected during the day, with the aid of tobacco smoke and a flashlight, in their resting places, such as tree buttresses, hollow trees, animal burrows, and leaf litter of the forest floor, low vegetation, rock crevices, holes in masonry, and to a very limited extent in Panama, in dwellings; at night, taken directly on human or animal baits or at lights, or in traps comparable to the well-known horse-baited stable trap, or in various types of light traps. In connection with certain items of equipment described in this report, their use in field collecting as well as in the laboratory is indicated.

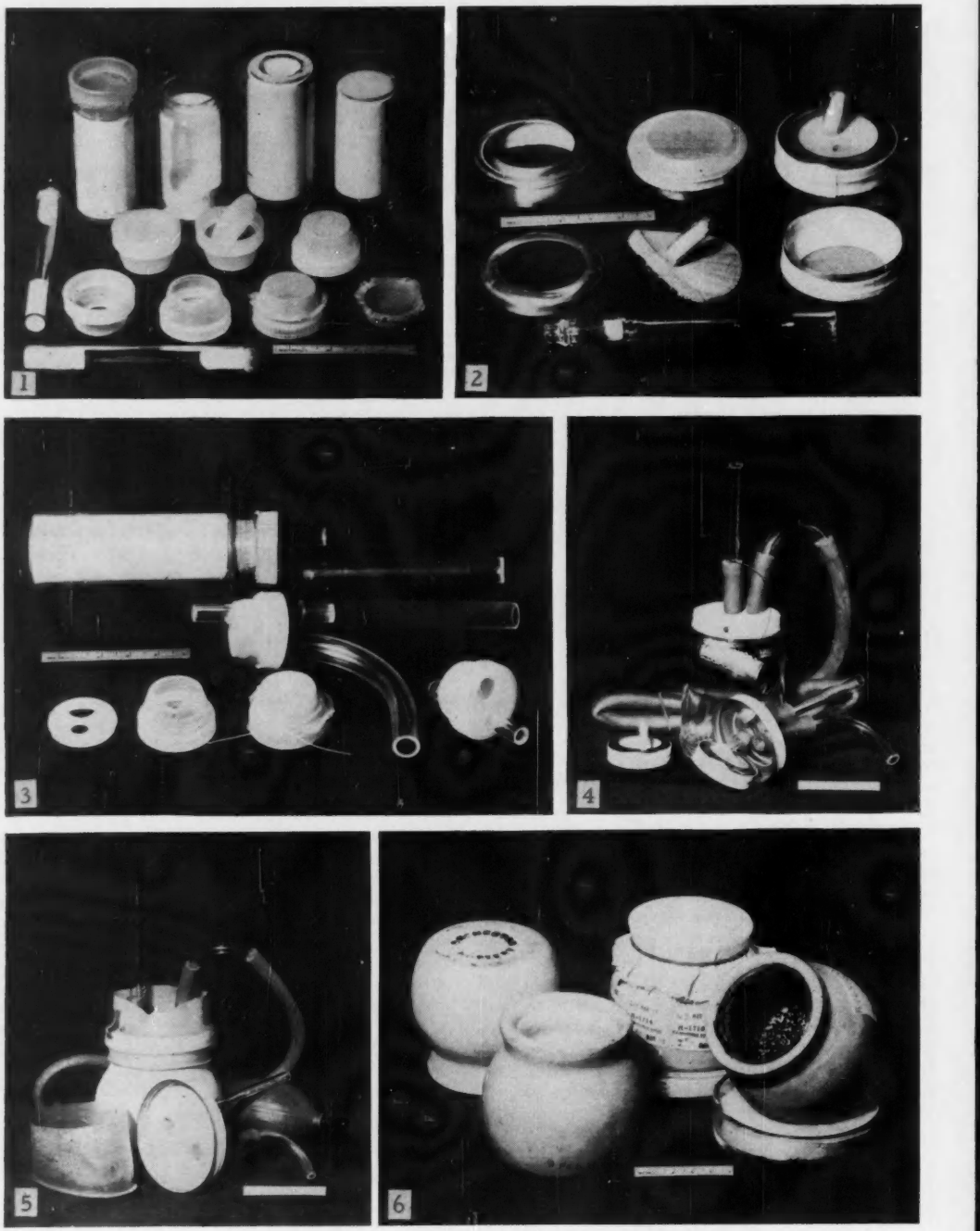
METHODS AND EQUIPMENT USED IN REARING *Phlebotomus*

Breeding vessels; larval food

The maintenance of the larvae throughout their relatively long period of development is the phase of the rearing process which has usually presented the greatest difficulties. Therefore the most important

[1] The work here reported was supported in part by a research grant (E-1251) from the National Institute of Allergy and Infectious Diseases, N.I.H., U.S.P.H.S. Partial cost of publication of this paper was met by Gorgas Memorial Institute of Tropical and Preventive Medicine, Inc. Accepted for publication July 7, 1961.

[2] Although not in accordance with the recommendations of the Committee on Common Names of Insects of this Society, this name was set solid in this paper at the request of the senior author.



FIGS. 1 to 6.—The small white scale is 5 cm. long.

items are the containers and the food for the larvae. The following are some of the combinations of breeding vessels and food which have been used by others:

Historical.—Earthen pot containing plaster block with earth and feces (lizard, rabbit or man); also tray in large cage (Waterston 1922); wooden box with garden soil plus lizard and other feces or insect bodies (Whittingham and Rook 1923); plaster block with central excavation containing pieces of stone and rabbit feces (Smith 1925); porous earthen vessel with earth and stable litter covered by a thick layer of dried mud, in which an opening gave access to the material beneath (McCombie Young et al. 1926); petri dish with filter paper, rabbit or goat feces, dried and ground (Christophers et al. 1926); porous earthen plate to which is transferred filter paper with eggs, rabbit or goat feces, at times enriched with dried blood (Shortt et al. 1926); plaster block or plaster-lined vessel, rabbit or goat feces with dried blood (Smith 1927). The above methods together with variations used later by several other workers were reviewed in some detail by Barretto (1942). He used chiefly porous earthen dishes. The larval food was soil mixed with material (mosses, lichens?) scraped from damp, shaded walls or the base of tree trunks, or soil rich in vegetable debris. He also found it desirable in the case of some species to "ripen" the food, a process used by Roubaud and Colas-Belcour (1927) and Ashner (1927), which consisted of holding the moistened mixture at 60° to 70° C. for a number of days.

In the above methods eggs were introduced either by transferring filter paper on which eggs had been laid or by confining the females in tubes, glass cylinders or by other means so that they laid their eggs directly in the rearing vessel or on the food material. During larval development the proper degree of moisture and humidity was achieved either by the direct addition of water, as in the case of filter paper in petri dishes, or, with porous vessels, by setting them in water or on moist cotton or sand. After pupation cloth covers, or tubes, glass cylinders and the like were arranged to receive the emerging adults, or the whole apparatus was placed within a cage.

Early in the work in China (Young and Hertig 1926) there were adopted as breeding vessels small unglazed earthen pots, lined with plaster of Paris, which served not only for the development of the young stages but also for holding live sandflies in the laboratory, securing oviposition and for the confinement of the emerging adults. These small pots, made for household use and obtained in the local markets, were "hard-fired" so that the degree of porosity was much less than that of ordinary flower pots, an advantage for our purposes. They were small enough (8 to 10 cm. in both diameter and depth) so that the interior could be inspected under the dissecting microscope. A lip permitted the easy fastening of a cloth cover with string or rubber bands. For the introduction of sandflies into the pots the silk bolting-cloth covers were provided with glass entry tubes. During larval development the pots were covered with earthen dishes, replaced after pupation by silk bolting cloth. The bolting cloth provided ventilation and permitted ready inspection of the adults. The plaster lining gave a white, porous surface, easily renewed. Also it was possible to judge the degree of moisture by the appearance of the plaster. Moisture was supplied by setting the thick base in water or on moist cotton. The larval food consisted of crushed feces of the Chinese striped hamster, which happened to be the most conveniently available source of rodent feces. A thin layer of food was sprinkled over the bottom of the pot shortly before hatching was expected, with more added from time to time as needed.

Essentially the same method was used in the case of *P. vexator*, a reptile-feeding species, collected at Plummer's Island, Maryland, and reared in Boston. The larval food consisted of dried guinea pig feces soaked in defibrinated horse or rabbit blood and then dried and ground. It was found that the size and shape of the small "individual-portion" size of the classic Boston bean pot made it ideal as a rearing vessel. They were made unglazed except for the lip, on special order, by the Dorchester Pottery Works, Dorchester, Mass. During the winter in the very dry air of the heated laboratory, it was desirable to enclose the pots and moist cotton in a more humid atmosphere, either under a bell jar or in cans with screw tops.

Porous Earthen Pots, Plaster-Lined.—These little

FIG. 1.—Plaster-lined vials, plastic stoppers modified to hold bolting cloth covers, for capturing and holding individual *Phlebotomus* sandflies, securing oviposition and rearing small lots. The four vials shown: 5-dram size ready for use; plaster lining partially removed; hole ground in bottom (7-dram size); bottom hole filled with plaster. Glass tubes, one end filled with plaster; useful for holding individuals of certain species.

FIG. 2.—Feeding cage; Monel metal frame, lined with plaster; bottom and cover of bolting cloth, the latter with glass entry tube; glass tube, bolting cloth screen in one end, for loading cage.

FIG. 3.—Plaster-lined vial (10-dram size) with plastic stopper modified to make an aspirating device for field collecting. The bottom of the stopper, partially cut away, is covered with bolting cloth, tied and cemented in place; the short entry tube has a removable extension; suction is applied through the removable curved tube fitted to a rubber tube.

FIG. 4.—Aspirating device for loading feeding cages; jar, metal cover with gasket; glass entry tube of feeding cage inserted in rubber entry tube of cover.

FIG. 5.—Aspirator top for rearing pot; metal cover fitted with entry and suction tubes; band cut from rubber glove holds cover in place with air-tight seal.

FIG. 6.—Rearing pots, unglazed earthenware, lined with plaster; moisture maintained by setting on cotton pads in petri dishes; newly established culture with larval food sprinkled on bottom and sides.

bean pots have been the standard rearing vessels throughout all subsequent work in Peru and Panama. The different batches of pots vary somewhat but the outside dimensions are approximately 80 mm. in height by 85 mm. in diameter at the widest part, 70 mm. at the lip, 64 mm. at the constriction below the lip, with the flat bottom 65 mm. in diameter (fig. 6). The walls and bottom are 7 to 8 mm. thick. The material is quite hard and only moderately porous, so that there is little danger of sudden changes in the moisture within the pot. The relatively large flat bottom provides not only considerable space for the larvae but assures good contact with the moist cotton on which the pot rests. The plaster lining is made by pouring into the pot, previously soaked in water and drained, a mixture of 50 cc. of dry commercial plaster of Paris in 25 ml. of water, rotating the pot to coat the walls and then allowing the rest of the plaster to flow to the bottom where it forms a layer about 8 mm. thick.

It should be noted that special types of plaster, e.g., that used by dentists, may contain substances (added for the purpose of modifying the setting characteristics) which are toxic to sandflies. In our experience, the plaster of Paris used by building contractors has been satisfactory.

The pots, with muslin covers, are autoclaved (20 minutes at 15 pounds) shortly before use. On discarding an exhausted culture the pots and bolting cloth or muslin covers are boiled and the pots washed with a brush. With repeated boiling and autoclaving and after several rounds of cultures the plaster lining becomes stained and the surface uneven. The old plaster when thoroughly wet is chipped and scraped out and replaced with a fresh lining. The porosity may in time become dangerously low. Such pots are either discarded or are marked and special attention given to make sure that the proper moisture is maintained, either by adding water directly to the plaster lining with a pipette or atomizer or by having the cotton pads frankly wet. The degree of moisture may be judged by the appearance of the plaster under the dissecting microscope, which must be learned by experience. The plaster should appear slightly "water-soaked" but a glistening film is to be avoided. The pads on which the pots rest are made by pressing cotton into petri dishes so as nearly to fill them (fig. 6). The petri dishes in turn are set in a stainless steel tray with water, which serves as a moat to protect against mites and ants. The moisture of the cotton is checked daily.

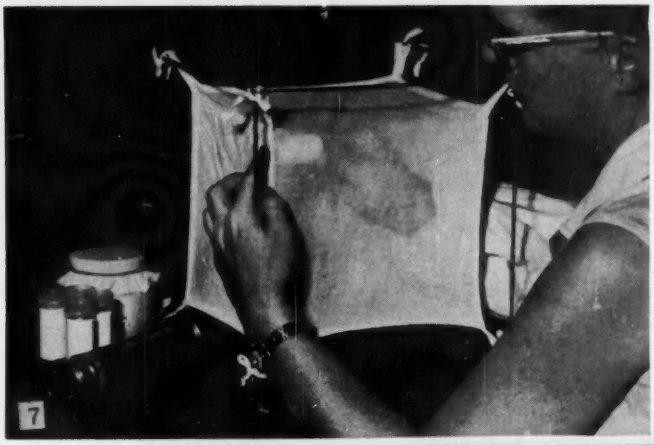
Larval Food.—A considerable number of substances in various combinations have been tried as larval food for our Panamanian species: rabbit, guinea pig, hamster, and sheep feces, alone or enriched by soaking in blood or adding powdered dried blood or commercial dried nutrient bacteriological media, dried yeast, decaying leaf litter and the underlying soil, green lichens on bark, ant-nest refuse, etc. While the larvae of various species developed well on the blood-enriched feces, the females thus reared were reluctant to take a blood meal. This may have been caused by the nutriment from the rich larval diet being carried over to the adult. Whatever the cause, the adults seemed to feed more readily without blood in the larval food.

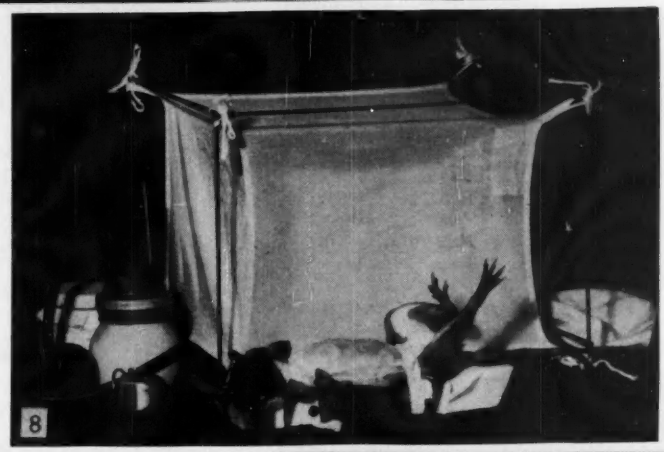
It was found in early trials that for some species it was desirable or even critical to have leaf litter present, whether the larvae fed on it or not. This was demonstrated by one dramatic instance. Newly hatched larvae of *P. nordestinus* were crawling away from the food (hamster feces plus dried sheep's blood) and climbing the bare walls of the pot. A small pile of well rotted detritus from under the leaves around a mango tree was placed on the bottom. Within 15 minutes some of the larvae had turned around and within 45 minutes a dozen or more were at the leaf debris. Thereafter they ate not the detritus but the food previously scorned. The 59 original eggs produced 56 adults. We have not had occasion to rear this species again nor have we observed any such striking behavior on the part of other species. However, this demonstration of the potential value of leaf litter led to its use as a standard ingredient of larval food.

At a time when the regular food consisted of rabbit feces soaked in blood, a mixture satisfactory for a number of species, we were having difficulty in rearing other species, such as *panamensis* and *trapidoi*. It was found that fresh green lichens on a chip of bark from the forest were attractive, especially to *panamensis*. At times almost all the larvae in a pot were concentrated on the lichens, actively "grazing." On the other hand, two burrow-inhabiting species showed no interest in the lichens. We were reluctant to introduce fresh material, with mites and other organisms, into the cultures as a routine, but this difficulty was avoided by the development of the standard larval food containing decaying leaves.

We have finally settled on the following as the standard larval food: (i) Dried rabbit feces, autoclaved, dried, ground and sifted, retaining that which passes a screen with 20 meshes per inch but is held by the 40-mesh screen; (ii) decaying leaves from the forest floor, autoclaved, dried, crumbled by hand, rubbed through a 15-mesh screen onto the 40-mesh screen; (iii) dried bodies of various types of insects, mostly Diptera, Orthoptera, and Lepidoptera, finely cut up with a knife on a board and similarly sifted. It is important to discard the portion passing the finer screen since the dustlike particles adhere to the larvae and they become exhausted trying to free themselves. The bulk of the food consists of equal parts by volume of the rabbit feces and leaves, to each half-liter of which is added 20 cc. of the insect bodies. The mixture is autoclaved and stored. Small quantities, sufficient for a day's needs, are placed in muslin-covered vials and autoclaved within a day or so before use.

The food is applied with a small spatula which is dipped in 95% alcohol and burned off before being inserted in the stock vial. Food is first given a day or two before the eggs are due to hatch, sprinkling





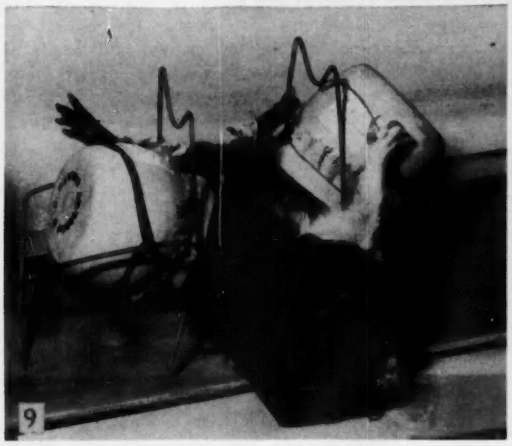


FIG. 7.—Small releasing cage (20 cm. cube) of muslin and nylon marquisette; the flexible material permits the free hand to aid in procedures within the cage.

FIG. 8.—Feeding sandflies on spiny rat, *Proechimys;* sandflies are released within the cage (medium size, 30 cm. long) and the immobilized animal, belly hairs clipped, is placed within; this in turn is placed under a frame covered with wet toweling.

FIG. 9.—Sandflies temporarily held in pots may feed directly through the bolting cloth covers; wire rack and rubber bands support two pots tilted against the clipped belly and sides of a guinea pig.

FIG. 10.—Apparatus for holding feeding cage in contact with animal's skin (spiny rat); cage is free to pivot on points of opposing set-screws.

a thin layer over the bottom of the pot and, for some species, part way up the walls, so that the larvae will not have to travel much more than their own length before encountering food, or if they have a tendency to climb, food will still be found nearby. Fresh food is added as needed. A culture with numerous older larvae may require fresh food every 2 or 3 days. We have considered it better to add food in successive layers sprinkled lightly over the older material, rather than give a large quantity all at once. In the case of certain species which are found on the surface of dead leaves in nature, such as *panamensis* and *pessoana*, small pieces of leaf, cut about 5 mm. square, are sprinkled over the regular food to give a more nearly authentic approximation of the natural environment.

Molds.—Molds have been found by most investigators to be troublesome at times. This is particularly true in a humid, tropical climate such as that of Panama. Nevertheless, a technique completely sterile throughout would probably not be desirable even were it feasible. Many of the fungi are in themselves palatable and they and other microorganisms probably form important parts of the larval diet. Actually it is not known whether any of the raw materials given as food would be satisfactory without the changes produced by the growth of microorganisms. The difficulties which molds cause are of two kinds:

a) The very young larvae may become entangled in the mycelia and never free themselves. This is especially the case with certain rapidly growing molds which produce a network of aerial mycelia. Usually by the end of the second instar the larvae can cope with this essentially mechanical problem. We have seen older larvae "graze" broad, clean areas in a thick, feltlike layer of mold. Heavy growths of mold may be removed with a needle which picks up a ball of mycelia as it sweeps through the growth. The aerial mycelia can also be pressed down into a mat, which presents fewer hazards for larvae, by the droplets of water from an atomizer.

b) Certain molds growing on the food are pathogenic to the larvae, notably a species of *Aspergillus* which appeared in our cultures for the first time about 2 years ago. This mold is dangerous chiefly when there is heavy growth. Apparently the larvae ingest spores which germinate in the intestine; the resulting mycelia invade the muscles of the thoracic region and kill the larvae. The persistence of this mold in our cultures necessitated a careful reexamination and revision of the procedures designed to prevent or reduce the introduction of microorganisms in general. Along with the measures outlined below, it was found that reasonable control could be secured with Mycostatin (Squibb Nystatin). A suspension in water, 2,500 units per cc., is sprayed into the cultures with a nasal atomizer. If the growth is heavy the pot must be sprayed every 2 or 3 days to prevent infection of the larvae. Since it seems that large concentrations of this antibiotic may be harmful to the larvae no more is used than absolutely necessary. Fortunately our strain of this *Aspergillus* seems not to have developed resistance to Mycostatin in the 18 months it has been used. There is no noticeable effect of this antibiotic on any other species of molds growing in our cultures. We have been able to confine this *Aspergillus* to cultures of certain sandfly strains which have been carried on for a number of successive laboratory generations and which we do not wish to lose. Autoclaving the affected cultures would probably eliminate the whole problem, but in the meantime another method gives promise of achieving the same result and saving these strains. Individual pupae are removed from an infested pot, washed several times in tap water and then placed in a clean, sterile pot.

Our current technique, some features of which along with strict control of all procedures are the result of the *Aspergillus* invasion, is designed to limit as far as possible the introduction of mold spores to those which inevitably accompany the parent females, either wild or reared, and to reduce the sources of air-borne contamination in the laboratory. The sterilization of pots and covers has already been mentioned; other types of containers and accessories used for collecting and holding sandflies and at times for rearing, are boiled or otherwise sterilized; the cotton pads and petri dishes are boiled each week and the trays serving as moats are scrubbbed clean. Work surfaces are wiped off daily and dust in the air-conditioned laboratory is held to a minimum. The food is autoclaved and whatever is left over after a day's work is reautoclaved. Any instruments touching the inside of a rearing vessel are sterilized by dipping in alcohol and burning it off, before touching the stock of food or any other vessel. The cloth cages used in transferring and feeding sandflies contain nylon panels and cannot be boiled and mere washing does not kill fungus spores. We found that cross contamination of cultures with the pathogenic *Aspergillus* could be reduced by fumigating the cages. After being used two or three times the cages are placed in a closed jar with crystals of paraformaldehyde, left at least 12 hours and then aired for 24 hours. As a result of these various procedures the larvae in most of our cultures are well established before the molds pose any real threat.

In the attempt to develop methods of mass culture we have had some success in rearing large numbers of larvae in glass-sided aquaria. The bottom of the aquarium was filled to a depth of 25 to 50 mm. with alternate layers of autoclaved leaf litter and finely sifted soil from the forest, with a layer of leaves on top. The culture was moistened and eggs, washed into a small beaker of water, were sprinkled over the leaves. In some cases standard larval food was sprinkled over the surface from time to time. Aluminum foil covered three sides and the top to give subdued illumination and the aquarium was set in a moat of water. This method produced fair numbers of adults of the five species tried, but the adults

were reluctant to feed either inside the aquarium or when transferred to a releasing cage. This general method is being followed up with trials of other types of rearing boxes and arrangements for feeding the adults without removing them from the cultures.

Maintenance of individual adults; oviposition

Up to and including the early stages of the work in Peru, the pots were used for securing oviposition and holding the adults for experimental or other purposes. There was then devised the glass tube filled at one end with plaster of Paris (Hertig 1940) which, with Peruvian species, admirably served a number of purposes (fig. 1). These tubes were 80 mm. long with a bore of 8 to 9 mm., with the plaster plug extending about 15 mm. into the tube and with cotton at the other end. They were made by standing a bundle of tubes in freshly mixed plaster. The proper degree of moisture (just short of condensation on the glass) was maintained by standing the tubes in moist porous pots or on damp cotton, or whenever necessary, by standing in water for a few moments. They were used routinely for collecting sandflies in the field and transporting them to the laboratory, and for holding individuals for oviposition or any other purpose. The eggs were usually laid on the moist surface of the plaster and were transferred by washing into the porous pots. Individuals could be kept alive in these tubes at times as long as 3 weeks.

On undertaking the rearing of Panamanian sandflies it was hoped that we would have the benefit of the extreme simplicity, economy, convenience and versatility of the plaster-plug tubes. However, it was soon found that our local species rarely survived 24 hours in these tubes. The precise nature of the difficulty was never determined. The commonest hazard, condensation on the glass in which the sandflies would become trapped, was avoided. Modification of the tubes by increasing the diameter or by coating the walls with plaster for about 15 mm. above the surface of the plaster base, for better footing for the sandflies, was equally unsuccessful. It was therefore necessary to devise other containers.

Plaster-Lined Vials.—A satisfactory solution was reached by using a relatively large glass vial and endowing it with some of the characteristics of the porous pots, namely, a plaster base with moisture entering from below, considerable space, walls completely covered with plaster, and ventilation through a bolting-cloth top. Kimble "Opticlear" vials with plastic stoppers have been successfully modified for this purpose (fig. 1). We have used several sizes, namely, 10-dram, measuring 80 x 29 mm., 5-dram, 55 x 27 mm. (no longer manufactured), and 7-dram, 65 x 29 mm. All three have the same sized opening, 21 mm., so that the stoppers are interchangeable. In all cases the inside is coated with plaster in much the same way as the pots, so that the walls as high as the shoulder, i.e., all but the 7 to 8 mm. occupied by the stopper, are completely covered with plaster, with a layer in the bottom about 15 mm. thick. A hole is then ground in the bottom of the vial 13 to 15 mm. in diameter, using a small electric grinder fitted with an abrasive cylinder about 10 mm. in diameter. Since the grinding of glass should be done under water and to avoid immersing the hands when using an electric appliance, a band of adhesive tape is wrapped around the base of the vial with enough projecting to hold a pool of water several millimeters deep. The hole in the glass and any excavation in the underlying plaster is later filled with plaster to insure good contact with the cotton or other moist surface on which the vials stand. In filling the hole in the bottom of the vial the plaster should be poured, allowed to harden and then trimmed with a knife, since any attempt to spread plaster with a spatula decreases the porosity.

The plastic stopper is used to make a frame for holding the bolting cloth cover (fig. 1). The stopper is hollow, forming a little cup covered by a removable plastic disk. The bottom of this cup is then cut out and the edges smoothed with emery cloth. With the hollow plastic cylinder thus formed a piece of silk bolting cloth, 40 mm. in diameter, is pressed into the vial. The bolting cloth in effect forms the bottom of the stopper, with an area for ventilation 18 to 19 mm. in diameter, through which it is possible to inspect the interior with a low-power lens or with the dissecting microscope. In preparing a batch of vials the bolting cloth disks are wet before pressing them into the vials; after drying they retain their shape.

Individual fed or gravid females are kept in these "KM-vials" on damp cotton in petri dishes or, with large lots, set directly on very porous earthen dishes which are in turn set in a tray of water which serves also as a moat to protect against mites and ants. A boiled raisin is placed on the bolting cloth of each vial. In view of the demonstrated, but physiologically unexplained, importance of boiled raisins in the experimental transmission of Indian kala azar (Smith et al. 1941) all our adult sandflies have constant access to boiled raisins as a convenient source of both fluid and nourishment, plus whatever benefits may accrue in relation to leishmaniasis. We have determined that the raisins definitely increase the longevity of our sandflies. The vials are inspected daily for the presence of eggs, most of which are on the bottom. A flashlight held against the vial gives sufficient light for this purpose through the thin plaster lining. After oviposition the females are identified and the eggs are washed into the porous pots. If the eggs are not readily dislodged, which tends to be the case with certain species, they may be gently touched with the end of the fine pipette used in the washing process. Eggs of the same species may be pooled up to 200 or 300 eggs per pot.

Rearing in Vials.—The plaster-lined vials serve a number of other functions. Individuals or small lots of adults of either sex may be maintained in them for various experimental purposes. They may also be used for rearing small numbers, such as the progeny of a single female. When no adults are

present the bolting cloth is replaced by muslin.

During ecological studies carried out by Mr. Wilford J. Hanson as a member of our group, 1957 to 1960, he recovered from natural breeding places a large number of larvae, of which several hundred were reared to the adult stage (Hanson 1961; see also Part II, table 1, Johnson and Hertig 1961). These larvae, unharmed by the screen-washing and sugar flotation technique, were reared in KM-vials with the standard larval food.

Field Capture with Vials.—One of the most important uses to which we have put these vials is the direct capture of fed females in the field, either with horse or human bait. Small carrying cases (aluminum lunch boxes, perforated for ventilation) are loaded in the laboratory with 25 to 50 vials, each complete with bolting cloth stopper. The vials are moistened in the field. The fed females are easily caught by placing the open vial over them as they rest on the horse, person or some nearby object. On being disturbed they fly into the vial and usually stay within so that the stopper is easily replaced. If the return to the laboratory involves a prolonged period in the heat of the following day, the vials rest on a wire rack with plastic bags of ice beneath. The sandflies are then maintained in the laboratory in their original vials, thus avoiding the labor and trauma to the sandflies involved in sorting and transferring the catch.

After use the vials and bolting cloth are boiled, while the plastic stoppers, which will not stand boiling, are dropped into 1:500 "Roccal" disinfectant, rinsed and then put into 70 percent alcohol. The vials are cooled gradually, and always under water, by allowing a small stream from the tap to flow into the hot water. Cooling in air increases greatly the danger of cracking. A certain amount of cracking occurs in any case, radiating from the hole in the bottom, the result of stresses set up in grinding. Cracked vials may be bound with adhesive tape and often survive repeated use and boiling.

Our first vials were of the 10-dram size but it was soon found that the smaller 5-dram vials (fig. 1) were equally satisfactory or even better for most purposes, particularly when examination was to be made with the dissecting microscope. With the discontinuance of their manufacture, however, we have been forced to use the 7-dram vials as the smallest size with stoppers which fit our general stock.

Aspirator Vial.—The large 10-dram vial, however, with the versatile plastic stopper transformed to make an aspirating device, is extremely useful in the rapid "bulk" collection of sandflies in the field and in some laboratory transfer procedures (fig. 3). We are indebted to our colleague, Dr. G. B. Fairchild, for the basic design of this apparatus. With a cork borer a hole is made in both the bottom and the removable top of the stopper to receive a short length (45 to 50 mm.) of ¼-inch methacrylate tubing, which serves as the entry tube; another hole is made only in the top to receive the ⅜-inch plastic tip of the suction tube. These holes are made slightly smaller than the respective tubes, so that the latter are firmly held by the elastic material of the stopper. Leaving support for the hole already made, as much of the rest of the bottom as possible is cut away. Bolting cloth, previously shaped to fit the stopper by being pressed wet into the vial and allowed to dry, is placed over the cut-out bottom, fastened with a clove hitch of stout thread, and coated with plastic cement wherever it is in contact with the plastic stopper. When the cement has hardened the entry-tube hole is cut through the bolting cloth with a cork borer, the cover is slipped back into its circular groove, the entry tube inserted to project equally above and below, and cemented in place. An 80-mm. length of ⅜-inch plastic tube serves as a removable extension when slipped over the ¼-inch entry tube.[3]

The aspirator tube consists of a convenient length (about 70 cm.) of rubber tubing fitted with a 100-mm. length of ⅜-inch methacrylate tubing with a right-angle bend and beveled for easy insertion into the appropriate hole in the top of the stopper. The short plastic tube can be smoothly bent by inserting each end in rubber tubing and suspending it in boiling water by the two rubber tubes held together. The only force necessary is supplied by the elasticity of the rubber; the ends of the plastic are protected against deformation. Suction can be applied directly by mouth or by using a rubber suction-pressure bulb. The latter is desirable particularly in dealing with large numbers of sandflies in the laboratory, to avoid inhaling scales, dust, etc. In the field each collector is provided with a number of aspirator vials plus a suction tube and an extension entry tube. The vials are moistened in the field before use. It is well to put no more than 15 sandflies in one vial if they are to be kept alive. The suction should be as gentle as possible since fed or gravid females are easily injured by too brusque an entry into the vial and, furthermore, there is inevitably some trauma from the agitation of the entire catch with each fresh entry.

Feeding adults

Virtually all species of *Phlebotomus* in nature suck blood only between dusk and dawn, although in dense forests some species occasionally feed also during the day. In the laboratory, however, the daytime feeding behavior varies greatly as between different species. In the early work on kala azar in India, *argentipes* in a releasing cage would feed on the arm of a patient only at night, although daytime feeding was later induced if the sandflies were kept in darkness. In our own experience certain species, e.g., *chinensis* and *mongolensis* in China, *verrucarum* in Peru, very rarely bite during the day either in nature or when liberated in a cage

[3] The series of sizes of methacrylate tubing commercially available, with outside diameters differing by ⅛ inch, have walls ⅛ inch thick and fit accurately over the next smaller size—a great convenience in various laboratory applications. It can be cut with a hack saw and the ends smoothed and beveled with an ordinary flat file, or for the inner edges, with a countersink. Methacrylate tubing is not yet generally listed in scientific supply catalogues; ours was obtained from Crystal-X Corporation, Lenni Mills, Pennsylvania.

with an animal nor do they attack the hand of the operator within the cage. Nevertheless, the same sandflies a few moments later when confined close to animal or human skin in a small feeding cage may feed at once.

In Panama the half-dozen principal forest species which attack man, horses, and other mammals, rarely bite in the daytime in nature and usually do not become active until after the brief twilight. However, in the laboratory two of these species, *sanguinarius* and *gomezi*, may feed readily in full daylight if liberated in a cage with an immobilized animal (fig. 8). They may also attack the operator's hand with such promptness and eagerness, even though the hand is in the cage for only a few moments at a time and is usually in motion, that it is necessary at times to wear a rubber glove as protection. The other species at best feed reluctantly and erratically. Night trials with some yielded no better results. Although there are unresolved difficulties in the feeding of these species, for the present at least, all our laboratory procedures are carried out during the day.

We have as yet made no attempt to determine the critical combination of factors which lead sandflies to leave their shelters and seek a blood meal, but it is obvious that this activity is usually preceded by a decrease in both light and temperature and a corresponding rise in humidity. In early trials we found that feeding results were improved by transferring the operation to another air-conditioned room with a slightly lower temperature. We then adopted the routine of manipulating the air-conditioning in the sandfly room so that the night temperature, 28° to 29° C., was somewhat higher than during the day, 26° to 27° C., thus reversing the conditions found in nature. As will be seen in the following descriptions of feeding operations, whenever there is prolonged exposure in a cloth cage, high humidity, necessary for sheer survival, is maintained in semi-darkness.

Releasing Cages.—Beginning with the work in China the releasing cages have in all cases been made of cloth, with at least two panels of some sort of netting, with no internal seams to obstruct visibility, and suspended by the corners in a frame. The first models were made entirely of silk bolting cloth, except for the muslin sleeve, and measured about 20 x 20 x 30 cm. Later a cubical cage with net internal dimensions of 20 cm. on a side, was found more suitable for routine releasing and sorting as well as for certain feeding operations. Later it was found desirable to have only two panels of bolting cloth (top and front) with the other panels of muslin giving a white, opaque background. A suitable grade of bolting cloth has about 20 meshes per linear centimeter. In Panama the 20-cm. cube with the same arrangement of netting and muslin panels is our standard releasing cage (fig. 7). In our current models, as a substitute for the silk bolting cloth, we have used nylon "marquisette," a fabric with the same weave as bolting cloth, i.e., the warp threads are double and pass on either side of the weft threads with a twist between each two, resulting in firmly locked square or rectangular meshes. Our nylon has about 16 x 22 meshes per linear centimeter. The finer nylon threads afford somewhat better visibility than the bolting cloth.

The cloth cages have various advantages over those with metal screen or other rigid material, quite apart from their extreme lightness and portability. The hand outside the cage enters actively into many operations. The finger tip on the outside of the cage aids in the capture of a sandfly when a vial or tube is placed over it (fig. 7). Containers may be firmly held by the free hand while corks, stoppers and covers are removed or replaced by the hand in the cage.

After considerable experience with different seamstresses and tailors, we have found it desirable to prepare the fabric ourselves. It is washed to preshrink it and remove sizing, and then with drawing board and T-square each seam, fold and cut is carefully marked; after cutting the folds are pressed with an iron. The muslin sleeve, 28 to 30 cm. long, is set into an opening 11 to 13 cm. square, the size depending on the objects, such as animal cages, which are to be inserted. The 6-mm. seams are double sewed with all cut edges "buried" within them and project flangelike at all edges of the cube, except that no seam is necessary between the two netting panels which are made of one piece. Even though it is possible to form the four muslin panels as one piece it is well to have the extra strength of the seams. The cage is suspended in its frame by two 20-cm. strands of tape sewed to each corner. With all seams on the outside there are no hiding places for sandflies except in the sleeve and relatively few seem to enter the sleeve. A rubber band holds the sleeve snug, but not too tight, around the arm. After withdrawing the arm the sleeve is brushed downward so that the surface presented to the inside of the cage is as nearly vertical as possible.

The cubical frame is made of ⅛-inch (3.2 mm.) brass rod, 25 cm. on a side, i.e., 5 cm. greater in each dimension than the suspended cage. To make the entire apparatus conveniently portable, the four vertical rods fit easily but not too loosely into 15-mm. lengths of brass tubing welded to the corners of the top and bottom parts of the frame. The tension of cage and tapes holds the frame together. The four loose rods are easily removed with the cage still tied to the corners of top and bottom; the whole apparatus can be collapsed for transportation and quickly set up again. (Like the cages themselves, the frames have passed through several developmental stages. At one time the removable vertical rods and the corresponding tubes were screwed together with right- and left-hand threads. The current arrangement is simpler and just as effective.)

Feeding Adults in Releasing Cages.—For the purpose of feeding sandflies liberated with an immobilized animal, two larger sizes of cage are in use. They are designed to accommodate an animal such

as a spiny rat, *Proechimys* (fig. 8), or a guinea pig. These cages are longer and broader but only slightly higher than the small releasing cages. The materials and general method of construction are the same. The inside dimensions are 30 x 25 cm. with a height of 21.5 cm. (fig. 8) and 40 x 35 x 21 cm. The sleeve is inserted at one end with an opening of 14 x 14 cm. In the larger cage three of the long panels, top, front and back, are of nylon marquisette while in the smaller one only the top and front are of netting (with the sleeve at the right). (In making a cage with the latter arrangement the needs of a left-handed operator should be considered; the small cubical cages with two netting panels and the larger one with three are suitable for either hand.)

The frames for the larger cages, made of the same brass rod, with four of the rods removable, have all dimensions 5 cm. greater than those of the corresponding cage. To support the weight of the animal if the cage is to be moved, a broad sheet of aluminum bent at the ends rests on the bottom end rods of the frame. For the purpose of maintaining a high degree of humidity during feeding operations, and also to provide semi-darkness, a large frame capable of accommodating any of the cages with their frames, is draped with wet toweling. The frame measures 56 x 51 x 32 cm. The cloth, blue Turkish toweling, is cut and sewed to form a boxlike hood over the frame, with one end hanging free. The top panel is supported by a rack made of coarse metal screening resting on the top of the frame.

In practice the animal is tied with wide gauze bandage, belly uppermost, on a narrow board (fig. 8). The belly hairs are removed with electric animal clippers. The animal is placed in the cage and the sandflies liberated from the breeding pots. The animal is free to move its head and legs but the cages are of such size that the cloth is rarely engaged by the animal's claws. After about an hour the animal is removed and the fed and unfed sandflies are transferred to separate clean, sterile pots, moistened and fitted with an aspirating device described below (fig. 5). About 20 fed females may be put into one pot, accompanied by an equal number of males, since copulation may not take place until after the blood meal. Depending on the numbers involved and the requirements for future operations, the unfed females and the rest of the males are either discarded or aspirated into other pots, with as many as 100 sandflies, females and/or males, to a pot. The aspirator tops must now be removed and replaced with bolting cloth without liberating sandflies in the process. The sandflies are immobilized by chilling the pots in a refrigerator at 4.5° C. for about 20 minutes. The exchange of covers is made, a raisin is placed on the bolting cloth, a muslin cover secured over all and the pots placed on cotton pads as in the routine handling of the cultures.

Aspirator Top for Pots.—The aspirator tops are made from the metal lids of glass jars, of such a size that they just fit over the top of the pots (fig. 5). (Lids of 6-ounce coffee jars happen to be about right.) Two holes are drilled somewhat smaller than the rubber tubing, about 10 mm. outside diameter, which will be squeezed into them to form the entry and suction tubes and which project about 25 mm. and 50 mm. respectively on the outside and about 5 mm. inside. The inner end of the suction tube is covered with bolting cloth; the entry tube is left open. The lid is held in place by a cylindrical band 50 mm. wide cut from the wrist of a rubber glove, which also makes an air-tight seal between lid and pot. When ready for use an 8-cm. glass extension is inserted in the entry tube, and the same aspirating tube with suction-pressure bulb, previously described, is fitted into the other rubber tube.

Feeding Through Bolting Cloth Pot Covers.—Females held in pots after having failed to feed when liberated may be fed directly through the bolting cloth covers. The arm of a volunteer (choosing one for whom the reaction to the bites is not severe) merely rests on one or two pots for about 15 minutes. For feeding on a guinea pig, which is the more usual practice, the animal is tied on its back with gauze strips and the hair clipped from the belly and both sides (fig. 9). A wire rack, with rubber bands, supports a pot on either side of the immobilized animal in such a way that the weight of the tilted pots holds the bolting cloth in contact with the skin.

Small Feeding Cages.—In feeding small numbers of sandflies some investigators have simply put them into test tubes and held the open end in contact with the skin. Throughout all our work we have used small feeding cages in which the sandflies, always within about 15 mm. of the skin, can feed through bolting (fig. 2). These cages are the lineal descendants of Wolbach's louse-feeding cage (Wolbach et al. 1922). In the early work in China the senior author used cages which had been presented to him by Dr. Wolbach. They were made from tinned metal ointment boxes with the top and bottom cut out and replaced by bolting cloth. To adapt these cages to flying insects the only modification necessary was to insert a glass entry tube in the bolting cloth cover. Later, to avoid corrosion, the sheet-metal stock was of Monel metal. The metal parts were made in commercial shops, the shaping being done not by stamping but by a process known as spinning, in which a revolving sheet of metal is gradually pressed over a form. The current models are 40 mm. in diameter by 14 mm. in depth, with openings in both top and bottom 29 mm. in diameter. Around the lower part of the bottom is a projecting "bead," over which the bolting cloth is fastened, made either by soldering in place a circle of wire or by having such projection "spun" during manufacture. The narrow rim of the bottom is pressed outward so that the bolting cloth can be drawn taut over its inner edge. (Otherwise sandflies would wedge themselves into loose places between metal and cloth.) The metal cover is made just large enough to slip snugly over the bolting cloth cover as the latter is pressed

into place. Wet bolting cloth is pressed over the bottom with a loose wire ring, bound over the projecting bead with a clove hitch of stout thread and allowed to dry. It is then trimmed and bound with a narrow strip of adhesive tape. The bolting cloth cover is shaped by being pressed on when wet and allowed to dry. To fix the entry tube (22 mm. long, of glass or plastic) in the bolting cloth cover, six star-shaped cuts are made, the tube is inserted to project about 8 mm. into the cage, the six points are bound to the tube with thread and then coated with plastic cement. The inner metal surface of the cage is coated with plaster of Paris to provide both moisture and proper footing for the sandflies. With the cover pressed on, a strip of adhesive tape around the cage holds it safely in place. It seems that the volatile substance which gives fresh adhesive tape its characteristic odor is toxic to sandflies, but the tape is safe to use after exposure in the air for several days. A stock of such "seasoned" strips is maintained on glass plates or other exposed surfaces.

Sandflies are transferred to the feeding cages by methods described below. The cages, usually with no more than 15 sandflies, are routinely held in contact with the skin of the host animal with gauze strips or by a device described below. Those sandflies which are ready to take a blood meal will usually have completed feeding within 15 or 20 minutes. The cages are opened in a releasing cage and the specimens transferred to appropriate vials or pots.

When feeding sandflies on human leishmanial lesions it is necessary to mask the lesion with aluminum foil, so that the sandflies have access only to the narrow border of indurated, unulcerated skin surrounding the central ulceration, this being the area where the parasites are concentrated.

Loading Feeding Cages.—The feeding cages are loaded by means of a transfer tube or an aspirating device. The transfer tube (fig. 2), made by a glassblower of two sizes of tubing, is 9 cm. long, the middle 5 cm. with a bore of about 4 mm., the 2 cm. at either end of a somewhat larger size, about 6 mm., which can slip easily over the entry tube of the cage. A bolting cloth screen is fitted in one end. The sandflies are caught one at a time by putting the open end over them as they rest on the sides of the releasing cage. Once inside the tube the finger is held over the open end and the fly makes its way or is shaken into the narrow portion, where its movement is restricted, since it cannot fly, and where it may be inspected with a handlens. Meanwhile the feeding cage, set in a slot cut in a pasteboard box, is held firmly with the entry tube horizontal. The cotton stopper is removed from the latter. When the sandfly is seen to be at a safe distance from the open end, the transfer tube is shifted quickly from the finger tip to the entry tube and the sandfly blown gently into the cage with a rubber bulb. (The first model of the transfer tube, devised in China, had a syringelike plunger which "nudged" the sandfly and caused it to run into the cage.)

Aspirator for Feeding Cages.—The aspirating device for loading feeding cages is made out of a glass jar with screw top of metal and an air-tight gasket (fig. 4). We have used the smallest available jar which will easily receive the feeding cage. It measures 68 mm. in diameter by 98 mm. in height, the inside diameter of the mouth being 47 mm. Two holes, about 9 mm. in diameter are drilled in the metal cover, one located so as to correspond with the somewhat eccentrically placed entry tube of the cage. Short lengths of rubber tubing, outside diameter 12 mm., are squeezed into the holes so as to project 30 mm. outside and 10 to 12 mm. inside. The inner segment of the rubber entry tube is wrapped with wire to insure a firm grip on the feeding cage's glass entry tube as the latter is pushed into place with the fingers. In use, the feeding cage hangs by its entry tube inside the jar. Outside, the rubber entry tube is provided with a plastic 8-cm. extension while the other rubber tube receives the suction tube previously described, which is used interchangeably for all our aspirator devices. After the sandflies have been aspirated into the feeding cage, the latter is removed by grasping the entry tube with rubber-tipped, curved haemostatic forceps and pulling it free.

Holder for Feeding Cage.—For feeding sandflies on Chinese striped hamsters, an apparatus was constructed in which the feeding cage was held by the sharp points of two screws set opposite one another in a metal ring, which in turn was fixed to a shaft free to move vertically in a tubular metal sleeve (fig. 10). The latter was held by an adjustable arm clamped to the side of the screen rack on which the animal was tied. This arrangement permitted the cage, resting on the animal's belly, to pivot on the two points and move vertically with the respiratory movements. The weight of the cage plus the movable metal parts was counterbalanced by rubber bands so that the pressure was just enough to maintain contact of cage with skin. A metal guard prevented the hamster's reaching the cage with its teeth. This apparatus is quite useful in the case of small animals which can be satisfactorily immobilized. We have used the original Chinese model with guinea pigs and spiny rats. These animals can easily bear the weight of the apparatus. Instead of being clamped to a fixed object it merely rests on the table, prevented from tipping over by a metal bar, such as a large file, inserted in the clamp (fig. 10).

Artificial Feeding.—For the purpose of infecting sandflies with *Leishmania* they were fed artificially a mixture of culture and defibrinated blood. The use of membranes gave only indifferent results, while the pipette method of Hertig and Hertig (1927) was very successful in an extended series of such feedings. These techniques and the related experimental work, in which a large proportion of the sandflies became infected, will be the subject of a separate report.

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The Rearing of *Phlebotomus* Sandflies (Diptera: Psychodidae) II. Development and Behavior of Panamanian Sandflies in Laboratory Culture[1]

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ABSTRACT

Observations on the immature stages and adults, in laboratory cultures, of 33 of the 65 known Panamanian *Phlebotomus* sandflies, of which 24 species have been reared to the adult stage, include the following: (i) General observations on larval food; temperature; feeding habits and other behavior of larvae; feeding habits of adults; copulation; fertility of eggs; longevity; diapause and quiescence. (ii) Detailed observations on the six principal man-biting species, notes on 27 other species, with tables showing numbers of eggs per female and the times of development from oviposition to adult and for individual stages.

We have been concerned with rearing Panamanian species of *Phlebotomus* as a part of investigations on leishmaniasis. We have been particularly interested in establishing the principal man-biting species in laboratory cultures for use in transmission experiments. Other species have been reared as opportunity presented itself, to settle taxonomic uncertainties in the matching of males and females and to provide immature stages for future taxonomic study as well as incidental biological information.

Eggs have been obtained from 33 of the 65 species known for Panama (63 described, 2 undescribed); 24 species have been reared to the adult stage and 4 more probably would have completed their cycle but were preserved as fourth instar larvae for taxonomic study. We failed to rear five species; in four of these the eggs hatched but the larvae died in the first instar, and in one the eggs did not hatch. Laboratory-reared females of 8 species have taken a blood meal in the laboratory, out of 11 species given the opportunity to feed on various hosts; 6 of the 8 were carried through at least a second generation, with 2 species, *sanguinarius* and *gomezi*, now in their 13th and 16th generations respectively (table 1). Most of the species dealt with are now being reared through at least one generation with relative ease.

The majority of the wild-caught females were freshly fed and were taken by our field crews on bait animals, mostly horses and themselves. Many of the wild-caught gravid females were collected by our colleague, Mr. Wilford J. Hanson, in the course of ecological studies (Hanson 1961).

The methods and equipment used in handling, feeding and rearing sandflies[2] are described in the preceding paper, Part I of this series (Hertig and

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[2] Although not in accordance with the recommendations of the Committee on Common Names of Insects of this Society, this name was set solid in this paper at the request of the junior author.

Table 1.—Panamanian sandflies, wild-caught: oviposition and rearing in the laboratory.

Species	No. of pools or individual ♀♀ ovipositing in laboratory	Condition when collected		No. of rearings to adult	Lab.-reared ♀♀ fed in laboratory	Stage attained	
		Gravid	Freshly fed, man, horse			Lab. generations	Not reared to adult
PRINCIPAL MAN-BITING SPECIES							
P. sanguinarius (Mar'57-May'59)	273 pools	+	+	258	+ (man, spiny rat, guinea pig)	13 (current)	
P. gomezi (Mar'57-May'59)	64 pools	+	+	52	+ (" " ")	16 (current)	
P. trapidoi (Mar'57-Oct'59)	135 pools	+	+	98[a]	+ (" " ")	2	
P. ylephiletor (Mar'57-Jul'59)	81 pools	+	+	64[a]	+ (" " ")	3	
P. panamensis (Mar'57-May'59)	187 pools	+	+	127[a]	+ (" " ")	2	
P. pessoana	36 pools	+	+	4[a, b]	0	1	
OTHER SPECIES							
P. aclydiferus	1	+		1		1	
P. apicalis	5	+	+	1		1	
P. arborealis	4	+		4		1	
P. barrettoi	3	+					Instar I
P. camposi	3	+		0[a]			Inst.IV,preserved
P. carpenteri	6	+					Inst. I
P. cayennensis	3	+		3		1	
P. cruciatus	1	+		1		1	
P. dysponetus	1	+		0[a]			Didn't hatch
P. galindoi	3	+	+	1[a]		1	
P. geniculatus	8		+				Inst.IV,prsvd
P. hamatus	1	+		1[a]		1	
P. hartmanni	2	+	+	1		1	
P. isovespertilionis	20[c]	+		20[c]		1	
P. nordestinus	1	+		1[a]		1	
P. ovallesi	5	+	+	4[a]		1	
P. pinealis	1	+					Inst. I
P. rubidulus	3	+		0[a]			Inst.IV,prsvd
P. runoides	1	+					Inst.IV,prsvd
P. serranus	3		+	2[a]	+ (man)	2	
P. shannoni	14	+	+	9		1	
P. spinosus	1	+		1		1	
P. trinidadensis	12	+		6[a]	+ (gecko)	1	
P. triramulus	2	+		2	+ (man)	1	
P. vespertilionis	23[c]	+		23[a, c]		1	
P. vexillarius	3		+	1		1	
Warileya rotundipennis	6	+	+				Inst. I

[a] Hanson (1961) reared 600 larvae, recovered from natural breeding places, to the adult stage, as follows: *panamensis* 8, *pessoana* 4, *trapidoi* 12, *ylephiletor* 2, *camposi* 1, *dysponetus* 24, *galindoi* 15, *hamatus* 437, *hansoni* 16, *nordestinus* 6, *ovallesi* 24, *rubidulus* 1, *serranus* 36, *trinidadensis* 13, *vespertilionis* 1.

[b] Including one very successful culture in a large aquarium.

[c] *Isovespertilionis* and *vespertilionis* can be identified only as adult males; 20 rearing attempts which were unsuccessful or produced only females are therefore not included.

Johnson 1961). The present paper (Part II) is concerned with the cycle of development and the behavior of the immature stages and adults of the various species which we have reared.

GENERAL OBSERVATIONS

Temperature.—On moving the sandfly cultures from an air-conditioned room maintained usually within a degree of 25.5° C., to another air-conditioned room with a temperature range averaging about 26.5°, it was found that certain species which were not doing well in the first room immediately began to thrive and the "hardier" species did even better at the slightly higher temperature. With this demonstration of the near-critical importance of a degree or two of temperature, we tried keeping cultures in various phases of development at the differing temperatures of rooms with and without air-conditioning. The following combination of temperatures has been found satisfactory for all our principal species.

The wild-caught females, in their individual plaster-lined vials, are kept in an air-conditioned room at about 25.5° until eggs are laid. While larvae do well at 26.5°, both hatching and larval development are more satisfactory at a still higher room temperature.

The rearing pots with eggs and larvae are kept in a room with open windows and through ventilation, with a daily range usually between 26° and 29°, with mid-day and afternoon temperatures occasionally reaching 30° or 31°. During the dry season, January through April, the relative humidity in this room ranges from 54% to 92%, but during the rainy season, May through December, the humidity is usually above 90%. When the adults begin to emerge the pots are transferred to an air-conditioned room maintained at about 26.6° during the day, but between 27° and 28.5° at night. The relative humidity during the dry season averages 77% at night and 71% during the day, with wet-season averages of 85% and 76% respectively. At the lower daytime temperature, a reversal of the conditions in nature, the adults feed more readily in the laboratory during the day.

In the latter air-conditioned room evaporation from the moat, the moist cotton and the pots themselves, lowers the temperature of all three so that the water averages 2.6° cooler than the air, and the food material in the bottom of the pots, with a range of 24.9° to 26.9°, averages about 1.0° cooler than the air. In the warmer open room with higher humidity these differentials are usually smaller, although there is considerable fluctuation. The interior of the pots with the eggs and larvae, ranging from 25.5° to 28.7°, average about 1.0° warmer than in the air-conditioned room.

Feeding Habits of the Larvae.—Most of the species we have reared in the laboratory have been observed ingesting molds found on the culture media as well as feeding on organic matter of the food proper. Frequently a larva will approach a colony of mold and remain without advancing for 24 hours or more while it swings from side to side eating a semicircular swath. This is particularly true of larvae of the slower-moving, surface-feeding species such as *ylephiletor* and *trapidoi*. A species of *Helminthosporium*, a mold first found in a pot of *gomezi* larvae, seems particularly attractive to *gomezi* and *ylephiletor* larvae and is eaten in preference to other molds in the pot.

Parts of insects are also utilized by the larvae. The five species we have dealt with most (*sanguinarius, gomezi, ylephiletor, trapidoi* and *panamensis*) are all particularly attracted to the soft, pigmented material on the inner surface of the eye capsule of Diptera.

Most of our species fall roughly into two classes according to their behavior in culture. (i) One group is always found on the surface of the food material, never burrowing under it. From this behavior we were able to predict that *panamensis, pessoana,* and probably also *trapidoi* and *ylephiletor,* would be found in nature on the surface of objects such as rotting leaves. This was confirmed for all four of these species by Hanson (1961) who found the larvae on dead leaves and debris scattered over the forest floor. The larvae of this group have long caudal bristles and the eggs are dark, thick-shelled, and have a sticky substance which presumably cements the eggs to the leaves or other forest litter, and thus prevents their being washed away by the rain. The larvae of *ylephiletor,* when very numerous in an aquarium culture, have been observed to consume all but the skeleton of decomposing leaves. Other litter- and leaf-dwelling species may also ingest material from the leaf proper although many times the larvae appear to be merely scraping off the leaf surface, presumably eating bacteria and fungi.

(ii) The second group of species burrow in the food mixture. Some are found with equal frequency above and below the surface of the food, as for example, *sanguinarius, trinidadensis* and *hartmanni,* while others, like *galindoi,* usually conceal themselves within or under aggregations of food particles, and do not occur on the surface. These species may have pale brown or black eggs, often with thin shells, which lack the adhesive substance occurring on eggs of *panamensis,* etc., and usually have the caudal bristles much shorter than those of the leaf- and litter-dwellers. Hanson (1961) found larvae of several of the species which have burrowing habits in culture in the top 2 inches of soil taken from between tree buttresses.

A few of the species we have reared do not fit either of the above categories. *P. gomezi* is a species with dark, thick-shelled eggs, but the larvae have short bristles and always occur on the surface of the food. *P. arborealis,* on the other hand, has very long caudal bristles but will crawl under the food.

Larval Reactions to Stimuli.—Most species show slight, if any, reaction to sudden increases in light intensity when the pot is opened under the microscope light. Neither the burrowing species, which presumably live in darkness in nature, including among others *sanguinarius, trinidadensis* and *hamatus,* nor the species which live exposed to constantly changing light intensities on upper surfaces of the leaf litter on the forest floor, such as *panamensis* and *pessoana,* appear to react to changes in light intensity. Larvae of *ylephiletor* and *trapidoi,* particularly the younger instars, may show increased activity and flick the caudal bristles for a few seconds after exposure to the microscope light. These species, which live as larvae in leaf litter next to the ground, are probably exposed to differences in light intensity mainly when the litter is disturbed, as by predators.

The usual response elicited by touching the caudal bristles of any species is a quick flattening backward of the bristles to the plane of the body. Usually the larva does not make avoidance reactions and we have observed that *geniculatus* and *panamensis* often will remain motionless for several seconds after such disturbance. *P. geniculatus* will also become completely motionless if water is dropped on it.

At times *ylephiletor* and *trapidoi* larvae near an area of the pot where mold is being removed with a dissecting needle will rear back with their heads near the tail end and with mandibles working. There may or may not be disturbance of the food

particles on which such larvae are resting. If this is a defensive reaction, it should be remarked that touching or pushing the larvae directly elicits no such response.

P. trapidoi, ylephiletor, sanguinarius, gomezi and *panamensis* have been observed to clean the caudal bristles and body hairs. The larva bends back dorsally, grasps the base of a bristle between its mandibles, and straightening itself out, draws the bristle through the mandibles. The process is repeated with the remaining bristles. Cleaning body hairs is accomplished in a random fashion, also with the mandibles. Since at times mold spores may completely cover a larva and its caudal bristles with no apparent effect on the larva and without stimulating any attempt at cleaning, the factors leading to bristle grooming are not understood.

Movement of Larvae; Places of Pupation.—*P. sanguinarius, triramulus, trapidoi, ylephiletor, galindoi, panamensis* and *geniculatus* are all slow-moving species and may remain in one place for extended periods. *P. gomezi, cayennensis,* and to a lesser extent *spinosus,* are constantly moving in the culture. *P. gomezi* and *cayennensis* are voracious feeders and, interestingly, are among those with the shortest life cycles of all the species we have reared.

Although all the species have a tendency to pupate on the side of the pot rather than on the food material or debris on the bottom, *panamensis, pessoana* and *triramulus* are particularly prone to crawl as far up the sides of the pot as possible before pupating. In the case of *sanguinarius, gomezi, ylephiletor* and *trapidoi,* climbing the walls by prepupal larvae is most common at the time pupation is just beginning while the later pupae often occur on the bottom as well as the walls of the pot. Probably in nature larvae have a tendency to crawl upward before pupating. This would be expected especially of burrowing species which would pupate in soil cracks or very near the surface of the soil in such a way that the adult could emerge from the pupal skin and get to the surface without injury. Within the artificial environment of the rearing pot the crowding of larvae may add an irritating factor which strengthens the tendency to travel upwards, particularly at the beginning of pupation when many larvae are still feeding and active.

P. sanguinarius larvae usually do not pupate in close proximity to each other when many are pupating at the same time. In a crowded pot, when first ready to pupate, larvae of this species may climb the sides in large numbers and end up desiccated and dead on the muslin top. This is the only species in which we have observed this suicidal mass migration. On the other hand, *gomezi* larvae in a crowded pot will oftentimes pupate at approximately the same time in groups of five or six, with the individuals all touching one another. *Panamensis,* though it pupates high on the sides, never goes so high that the larvae or pupae die from desiccation.

Feeding Habits of the Adults.—Females released in a cloth cage containing an animal or a human hand do not react in a constant manner, even females of the same species. At times they begin to feed immediately in the full light of the laboratory; other groups will feed sporadically over a period of an hour while the cage is covered, and thus darkened, with a wet towel; others show the most hunger when the operator removes the wet towel and begins to aspirate the flies into a pot. Sometimes the females refuse to feed at all. In general, a higher proportion of the females feed if they are held until 6 or 7 days after the first adults emerge in a given culture.

We assume that the change from the temperature of the rearing pot to that of the room, the relative humidity in the laboratory, the age of the females and the disturbance incidental to releasing the adults, all play some role in affecting the feeding response. Probably a drop in temperature and corresponding rise in humidity help to provide the stimulus to feed in nature, since these conditions usually prevail during the time sandflies are active and searching for a blood meal. However, the relative humidity of the laboratory, which ranges from 65% to 95% depending on the season, is usually too low to allow survival of adults in cloth cages for more than a few hours unless protected with wet towels. Even so, adults released from a pot with a relative humidity approaching 100% may begin to feed immediately at the lower humidity of the laboratory. The mere fact of a change in the environment, i.e., in light intensity, temperature or humidity, may lead to the feeding response in physiologically hungry females.

Hungry females are usually restless, flying from place to place in the cage and thus exciting the other sandflies, possibly inducing more to feed. A female may insert the piercing stylets three or four or more times in different places on the skin before settling down to feed. The time needed for engorgement differs with the individual female. Some hungry *sanguinarius* and *gomezi* females may feed to repletion in less than a minute, others take as long as 5 minutes.

P. sanguinarius and *gomezi* are our hardiest species and the females are the easiest to feed in the laboratory. They feed readily on man, guinea pigs, or the spiny rat, *Proechimys,* whether in feeding cages or free in a releasing cage. They attempt to bite hamsters but these animals are so difficult to immobilize and react so promptly to the sandfly bite that feeding is rarely successful. When immobilized with drugs hamsters seem to lose their attractiveness for sandflies, possibly because of a drop in skin temperature. By keeping the hamster warm in the operator's hand some feedings have been successful. Various attempts to feed *sanguinarius* and *gomezi* on suckling white mice all failed. *P. ylephiletor, trapidoi* and *panamensis* do not feed readily in the laboratory on any of the animals or under any conditions so far tried.

Copulation; Fertility of Eggs.—We have found that to assure enough fertilized eggs to maintain and increase our laboratory colonies, males must be placed in pots with fed laboratory-reared females,

which indicates that copulation in the laboratory often does not occur before the blood meal.

In the laboratory *sanguinarius* females have been observed in copula before feeding, when freshly fed, and when the blood meal was 2 days old. At times for reasons not known, many of the *sanguinarius* adults released from a pot in a cloth feeding cage will immediately pair. Only three times has *gomezi* been observed in copula, once in a pot containing unfed flies, and twice in a releasing cage (one fed female, one unfed). In the laboratory many flies apparently do not copulate at all since the fertility of eggs laid by laboratory-reared females is lower than that of wild-caught females. Probably in nature *sanguinarius* and *gomezi* usually copulate before feeding since the fertility of the eggs of wild-caught females taken in the act of feeding and maintained without males, is high. Of 128 wild-caught *gomezi*, all but six laid fertile eggs. Eggs from wild-caught *sanguinarius* also have a high fertility. Twenty pools, each containing 1 to 10 females, gave the following proportions of eggs hatching and developing to the pupal stage: 9 pools, 90%-100%; 2, 80%-90%; 3, 70%-80%; 1, 60%-70%; 4, 50%-60%; 1 pool (eggs from a single female), 20%-30%.

In another experiment, four groups of *sanguinarius* females, representing wild-caught and tenth laboratory generation populations, laid fertile eggs as follows: Group A, 23 wild-caught females maintained without males after feeding; 92% of all eggs hatched. Group B, 33 tenth-generation females maintained with males before, during and after feeding; 23% hatched. Group C, 19 tenth-generation females maintained without males after feeding; 15% hatched (as opposed to 92% in the case of eggs laid by wild-caught females maintained under similar conditions). Group D, 10 tenth-generation females maintained with males before but not during or after feeding; none of the eggs hatched. An interesting supplementary observation was that although the average number of eggs laid by Group B females was 43, Group C laid an average of 28 eggs, and Group D only 19 eggs.

Search for Index of Previous Blood Meals.—Some of the wild-caught females in our series probably were feeding for the second or third time and may have copulated after the first feeding with sperm being conserved in the spermathecae. At the present time we do not know what proportion of our wild-caught females have fed more than once. Preliminary investigations have failed to establish a satisfactory index of prior feeding. It has been considered that all sandflies which had granules in the accessory glands of the ovaries had taken a blood meal at some previous time (Adler and Theodor 1935). Lewis and Minter (1960), following extensive investigation and dissections of wild-caught African sandflies, stated that usually a fly may be considered unfed and nulliparous if there exists a combination of no remnant of a blood meal in the gut and no or few granules in the accessory glands of the ovaries. Unfortunately neither wild-caught nor laboratory-reared specimens of those Panamanian species for which we have the most information follow this pattern. The granules may be present or absent both before and after feeding; they may be in one gland and not the other; there may be active contraction and the granules are often evacuated. Lewis (1960) anticipated that this difficulty might exist with regard to the Central American species of *Phlebotomus* based on the finding by Garnham and Lewis (1959) that a surprisingly high proportion of wild-caught Honduran sandflies had granules in the accessory glands.

Longevity of Adults; Refeeding of the Females.—In the laboratory most females die after laying their first batch of eggs, with those that live past oviposition refusing a second blood meal. Refed females will live longer than females feeding only once. We have obtained refeeding, after egg laying, in *gomezi, sanguinarius, ylephiletor* and *trapidoi*; one *sanguinarius* female fed four times in all. The longest periods of survival (after the first feeding or offer of a blood meal, initial age unknown) for refed females and unfed females respectively, are: *gomezi* and *sanguinarius*, 19 and 14 days; *ylephiletor*, 17 and 12; *trapidoi*, 18 and 10. Males lived as long as the unfed females of their respective species.

In our laboratory, females in the presence of males both before and after refeeding laid eggs which were more often than not infertile and the larvae which did hatch had a low viability. Refed females maintained without males after the first feeding rarely laid fertile eggs. In only one such batch of eggs (from a refed *sanguinarius* female) did hatching occur. Dissections of females which had laid eggs previously in the laboratory rarely revealed sperm in the spermathecae. Whether this is an artificial phenomenon caused by laboratory conditions remains to be investigated.

Adler and Theodor (1935) stated that wild-caught *papatasi* may refeed several times with no relation to egg laying whereas *perniciosus* takes one blood meal and refuses to refeed until a batch of eggs has been laid. They also found that gravid *perniciosus* females need a higher humidity than do those of *papatasi* in order to lay eggs, and suggested that *papatasi* maintains its water balance by taking frequent blood meals.

There are indications that in nature certain Panamanian species may take additional blood meals between the primary meal and egg laying. In dissections of over 700 wild-caught females, taken from animal and human bait before or during feeding and dissected within 24 hours of capture, five females showed evidence of having fed within 3 or 4 days. In four cases, two *sanguinarius* and two *trapidoi*, females had what appeared to be the remains of an old blood meal without discrete red cells in the midgut, but there was no ovarian development. The fifth female, *sanguinarius*, had taken a small fresh blood meal but the ovaries had begun to develop previously. Further evidence is the following:

(i) Ten laboratory-fed females of *panamensis* all laid eggs beginning the fourth day after feeding. However, one or more of the females in 3 of 127 pools of wild-caught females laid eggs 1 and 2 days after feeding, while in the other 124 pools egg laying began after 3 to 5 days. The indication is that the females which laid eggs only 1 or 2 days after a blood meal probably had fed previously and the eggs were the result of the earlier blood meal.

(ii) Females of 2 of 64 pools of wild-caught *ylephiletor* laid eggs 2 days after feeding and the other 62 pools laid eggs after 3 to 7 days. Of 13 pools of laboratory-reared *ylephiletor*, only 2 laid eggs 3 days after feeding while the rest laid eggs after 4 to 8 days.

(iii) One wild-caught female of *gomezi* laid eggs 2 days after feeding. This female did not produce autogenous progeny and possibly she too had taken an additional blood meal before oviposition.

(iv) No wild-caught *sanguinarius* females have ever laid eggs within 2 days after feeding, nor have laboratory-reared females. Wild-caught females in only 2 of 258 pools laid eggs after 3 days, while all the others laid eggs 4 to 10 days after feeding. This may be an indication that *sanguinarius* normally does not feed more than once before laying eggs, even taking into account the fact that this species is often slow in ovarian development.

Diapause and Quiescence.—There are many reports of diapause in the fourth larval instar of Palaearctic species of *Phlebotomus*, with over-wintering achieved in this manner. This may be considered true diapause in which the larva enters a prolonged arrest of development which is genetically determined and independent of environmental factors. For example, laboratory-reared *P. chinensis* may go through two or three uninterrupted generations and then enter diapause in the third or fourth generation, during which the fourth-instar larvae may continue to eat but do not pupate. This diapause lasts at least 6 to 8 months in nature but in the laboratory the diapause is shortened by about 2 months. Of course, in nature diapause occurs during the cold winter months. In the laboratory, with a diapause of shorter duration, successive generations eventually get out of phase with the external environment and diapause will occur during summer, the most favorable natural period.

In the laboratory, adverse culture conditions such as too much moisture or a lowered atmospheric temperature may cause fourth-instar larvae of the Palaearctic species to enter a quiescent period (Whittingham and Rook 1923, Adler and Theodor 1957). Addis (1945), working in Texas with the Nearctic *P. anthophorus*, was unable to obtain pupation of fourth-instar larvae over a period of 2 months when using a mixture of dried rabbit feces and blood as larval food. He did not give the time of the year involved. When he added a few drops of all the known components of the vitamin B complex to the culture, the larvae pupated within 3 days. Later Addis used rich garden soil and rabbit blood and pupation occurred normally in his cultures. Possibly Addis was dealing with the same type of quiescence which is seen in Palaearctic species maintained under adverse conditions.

Barretto (1942) observed diapause in fourth-instar larvae of a Brazilian species, *P. whitmani*. While this phenomenon was rare in his laboratory, Barretto had certain cultures of *whitmani* in which as many as 47.6% of the larvae entered a diapause which lasted 96 to 203 days. According to Barretto's account this was a true diapause, since it was independent of environmental conditions of temperature, humidity, and food. It occurred in larvae reared from eggs laid by both wild-caught and laboratory-reared females.

Lindquist (1936) made an important and seemingly overlooked observation regarding diapause in Nearctic *P. diabolicus* from Texas. He found that eggs of *diabolicus* maintained in his laboratory diapaused 167 days, from 14 October 1933 to 29 March 1934. Lindquist suspected that the fourth-instar larvae of *diabolicus* might also overwinter.

In our tropical species we have indications that quiescence may involve not only the fourth-instar larvae but also the eggs. Quiescence in fourth-instar larvae of the Panamanian species probably takes place under adverse conditions only, with lack of moisture being the most important factor. In one culture of *panamensis*, which had produced adults and had then been overlooked and allowed to become too dry, fourth-instar larvae were still present in the absence of pupae and living adults 2 weeks after adults first appeared. This does not occur in a normally producing pot where adults and pupae are invariably present as long as there are still developing larvae in the pot. When the pot in question was again moistened, some of the larvae which had been motionless and desiccated in appearance, and with food visible in the gut, continued development, pupated, and produced normal adults. Others of these larvae died without pupating although they were alive at the time the culture was moistened.

In one culture of ninth laboratory generation *sanguinarius* the same situation as with the *panamensis* larvae was observed. Adults first emerged from this pot 55 days after oviposition. A month later (at 86 days after oviposition) the pot was very dry and contained one slowly moving fourth-instar larva with food in the gut, one pupa, and no living adults. The pot was watered and the pupa emerged at least 92 days after oviposition. The fourth-instar larva pupated but was not followed beyond this point.

There are extremely uneven rates of hatching time and larval development in some of the Panamanian species. *P. gomezi* may have all stages from the second larval instar through the pupa present in a culture established with eggs laid within a 2-day period. The same is true of *panamensis* although usually to a lesser degree. In several cultures of *panamensis* which were started from eggs laid by wild-caught females during the dry season, the first hatching took place within 9 to 10 days, but 3 or

4 weeks later, with most of the larvae in the fourth stage or already pupae, newly hatched larvae were discovered, i.e., at least 30 days after oviposition. Further, in a culture of *geniculatus*, a species related to *panamensis* and probably with the same habits in nature, a newly hatched first instar larva was observed 36 days after oviposition. Since other larvae in these cultures of *panamensis* and *geniculatus* had hatched at normal times culture conditions probably were not involved, and some eggs may be destined to be quiescent.

P. sanguinarius has a uniform rate of larval development with usually no more than two stages of development present at any one time in a single culture, and probably either no quiescence occurs in the egg stage or our laboratory conditions have never induced such quiescence. As noted earlier, *panamensis* and other surface feeders in culture, including *gomezi*, have eggs with thick, black shells, and *sanguinarius* eggs have thin, light-colored, easily collapsible shells. As well as furnishing protection against drying during the normal prehatching time of 5 to 12 days, depending on the species, the resistant shell probably serves as protection during periods of egg quiescence.

In Panama, unless the year is exceptional, occasional rain falls during the dry season, especially on the Atlantic side of the Isthmus. Because periods of drying would not necessarily be extensive, eggs of our species might not be geared to a lengthy and true diapause, a possibility which has not been tested in the laboratory. *P. panamensis* is not very abundant during the dry season, but in June, i.e., a month after the rains begin, there is a rapid increase followed by a decline at the end of June. The related species *pessoana* has a similar but lower June curve.

We routinely discard batches of eggs which do not hatch within 21 days, and longer periods of quiescence involving all the eggs of a given female or pool of females might occur without our knowledge. However, at the end of the 3-week period most unhatched eggs are either shrivelled or moldy and obviously not viable. Furthermore, eggs of *sanguinarius, gomezi, ylephiletor* and *trapidoi* have not been observed to hatch between days 17 and 21 after oviposition.

Autogeny.—One Panamanian species, *gomezi*, has occasional autogenous individuals in nature, and several autogenous strains have been maintained in our laboratory, with one of them attaining the 11th laboratory generation (Johnson 1961). Autogeny has not been demonstrated in the other species we have reared in successive laboratory generations.

OBSERVATIONS ON INDIVIDUAL SPECIES

We have reared large numbers of *sanguinarius, gomezi, trapidoi, ylephiletor* and *panamensis* and our observations on these species are extensive. The other Panamanian species were reared only as fed or gravid females became available. The number of such rearings is relatively small and observations on the laboratory development are correspondingly fragmentary. Although we have as yet made no attempt at taxonomy of the immature stages, the larvae of our common species or species groups can be identified from both morphological characters and behavior in cultures. Therefore in the following accounts, appearance of the larvae and pupae is sometimes mentioned. Conspicuous features of the larvae of all species are the caudal bristles. They may be longer than the body and project fanlike behind or are held erect. In general there is a single pair in the first instar but two pairs thereafter. The *Brumptomyia* group, however, has only a single pair in all four instars.

The average numbers of eggs per female as given in table 2 for the various species are probably low since figures from our early rearing efforts are included. Those cases in which only one or two eggs were laid probably represent the dying efforts of females in extremis and bear no relation to the number which would otherwise have been laid. With the improved methods of the past 2 years for maintaining wild-caught females the number of eggs per female has increased. For example, in one group of 23 *sanguinarius* collected in late 1960, the average number of eggs per female was 39, compared with 27 as given in the table.

Table 2.—Number of eggs laid by individual wild-caught females; principal man-biting species.

	sanguinarius	*gomezi*	*trapidoi*	*ylephiletor*	*panamensis*	*pessoana*
Number of ♀♀	2,268	377	1,103	449	1,215	147
Average no. eggs per ♀	27	29	21	27	28	20
Extremes	1 - 80	1 - 72	1 - 75	1 - 92	1 - 90	2 - 55
Extremes of middle 90%	3 - 55	5 - 53	2 - 45	2 - 63	3 - 57	5 - 42
Extremes of middle 50%	16 - 38	18 - 32	9 - 31	13 - 37	16 - 42	11 - 28

PRINCIPAL MAN-BITING SPECIES

P. sanguinarius F. & H.—This is the most common species in our biting collections from man and horse. In the laboratory, wild-caught females laid an average of 27 eggs, which closely approximates the averages for *gomezi, panamensis* and *ylephiletor* (table 2). The extremes and the curve of number of eggs per female are also approximately the same for the above species. The period of development of *sanguinarius* from oviposition to adult emergence averages about 50 days and is the longest of the common species we have dealt with. (Tables 3 and 4.)

Table 3.—Shortest number of days, oviposition to adult emergence, in successive laboratory generations; principal man-biting species.

Laboratory generation	*sanguinarius*			*gomezi*			*trapidoi*			*ylephiletor*			*panamensis*			*pessoana*	
	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes
1st	40	44–53	49.3	106	28–43	33.8	39	38–54	42.9	21	34–48	40.3	27	30–45	36.4	4	32–38[a]
2nd	21	45–59	49.0	9	29–45	36.0	4	44–49	46.0	7	37–49	41.3	3	32–48	40.7		
3rd	22	45–63	51.0	10	29–39	32.5				3	40–47	43.0					
4th	23	47–55	51.1	9	28–36	32.7											
5th	20	47–54	50.0	10	31–42	34.8											
6th	9	47–56	51.4	13	31–38	36.0											
7th				10	30–41	35.3											
8th				8	34–41	37.0											
9th	4	44–50	47.5														
10th	5	48–55	49.0														
13th				4	31–36	34.3											
14th				3	36–40	38.3											

[a] Exact number of days known for only one of the four cultures.

The egg of *sanguinarius* is oval, brown in color and the shell is thin, often collapsing after hatching. The larvae have black heads and pale-buff bodies. The caudal bristles are short and the larvae are found with equal frequency burrowing in the food or on its surface. The larvae are slow-moving and are not noticeably affected by a change in light intensity although the pupa at times performs a bowing movement when exposed to a sudden increase in light. Although there may be a great many larvae in a rearing pot, they usually are not disturbed by each other and flourish if enough food is present. As the time of pupation approaches, the larvae climb the sides of the pot. In a crowded pot they often crawl onto the cover of the pot and almost immediately become desiccated. It has been noticed that sometimes the mass migration will occur only on a certain area of the pot wall, which may be covered thickly with larvae all slowly moving upward toward the rim, while the other parts of the wall have relatively few. In an aquarium culture consisting of crumbled soil not mixed with leaves, *sanguinarius* usually pupated at high points of the surface although some pupae were found at lower levels of the soil surface. Larvae in the aquarium culture were seldom seen and presumably were below the soil surface.

Tables 3 and 4 show that the successive laboratory generations of *sanguinarius* do not differ significantly in total number of days from oviposition to adult emergence nor in number of days per individual stage. Experiments suggest that the average number of eggs laid by individual females of the 10th laboratory generation, 43 eggs per female in one experiment, is significantly higher than for wild-caught females, which have laid an overall average of 27 eggs (39 under later improved conditions) although the fertility of the eggs from the 10th generation may be very low. Despite this difficulty, our 10th and succeeding generations are well adapted to the laboratory and females take blood meals in satisfactory numbers.

P. gomezi Nitz.—This is our most rapidly developing man-biting species. Total time from oviposition to adult emergence averages about 34 days (table 3). All stages of the life cycle take less time than in *sanguinarius*, especially the egg, the fourth-instar larva and the pupa (table 4). The average number of eggs laid by individual wild-caught females, 29, is about the same as in *sanguinarius* (table 2).

Table 4.—Shortest number of days per stage, principal man-biting species.

Species, laboratory generation	Blood meal to egg			Egg			Larval instars												Pupa		
							I			II			III			IV					
	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days	No. cultures	Extremes	Avg. days
sanguinarius																					
1st	44	3–10	4.1	45	9–13	11.0	55	5–9	6.0	55	2–5	3.6	54	3–7	4.8	52	7–12	9.7	50	12–16	14.2
5th	18	4–7	4.7	20	10–14	11.5	20	4–7	6.0	20	3–6	4.3	20	3–7	5.0	20	8–14	11.0	20	11–13	12.2
10th	4	4		3	10–11	10.3	3	4–7	5.7	4	3		5	2–6	3.4	5	10–16	13.8	5	11–14	12.0
gomezi																					
1st	121	3–10	5.1	82	6–14	8.2	105	3–8	4.8	106	2–6	3.3	94	2–8	4.1	87	4–10	6.4	69	5–9	7.0
5th	9	3–5	3.4	10	7–10	7.7	10	4–7	5.4	10	2–6	4.0	10	3–6	4.0	10	5–9	6.6	10	6–9	7.1
14th	4	3–4	3.8	4	8–10	8.8	4	6–8	6.8	4	4–8	5.3	4	1–4	3.0	3	6–8	6.7	3	7–10	8.7
trapidoi																					
1st	41	3–5	4.0	47	8–14	10.8	50	4–9	5.9	49	2–7	3.9	48	3–8	4.7	48	5–15	8.8	38	7–14	8.5
2nd	4	4–5	4.3	4	8–15	12.0	4	4–9	5.5	4	4–5	4.3	4	5–7	5.8	4	9–15	11.5	4	7	
ylephiletor																					
1st	24	2–7	4.6	24	7–13	9.5	22	5–8	5.7	24	3–6	3.8	22	3–6	4.9	20	4–12	7.9	20	7–16	8.4
2nd	6	3–6	4.7	7	7–10	8.9	7	4–7	5.6	7	3–4	3.7	7	3–6	4.9	6	7–15	9.8	7	7–9	7.7
3rd	3	4–8	5.3	3	9–10	9.3	3	5–8	6.3	3	3–6	4.0	3	5–6	4.3	3	8–12	10.0	3	7–9	7.7
panamensis																					
1st	35	1–5	3.6	35	8–12	9.8	35	3–7	4.9	34	2–5	3.2	28	2–6	3.8	27	4–12	7.6	25	3–9	6.6
2nd	1	4		3	9		3	6–8	6.7	3	2–5	3.0	3	5–8	6.0	3	3–19	7.3	3	4–7	4.3
pessoana																					
1st	20	3–7	4.1	20	8–12	9.6	5	5–8	6.0	4	4–6	5.3	1	3		1	9				

The eggs are oval, black and thick-shelled. The larva has short caudal bristles, a pale brown head, and a very pale, almost translucent body. The larvae are extremely active and apparently are not affected by changes in light intensity. They remain on the surface of the food, never burrowing under or into it. Like *sanguinarius* and other species, the larvae tend to climb the wall of the pot in order to pupate.

Successive laboratory generations take a variable number of days for development (tables 3 and 4). One strain which is now in the 16th laboratory generation is vigorous and well adapted to laboratory conditions.

In a comparison of the number and fertility of eggs of early and advanced generations of *gomezi*, 11 second-generation females laid an average of 49 eggs, 85% of which hatched, while 10 14th-generation females laid an average of 55 eggs, only 49% of which hatched. In an earlier section we discussed the possibility that in the older laboratory generations copulation is not so frequent, for unknown reasons. The increase in number of eggs per female with succeeding generations probably reflects their adaptation to laboratory conditions.

P. panamensis Dyar.—This is a litter- and leaf-dwelling species in nature. In length of life cycle it is intermediate between *sanguinarius* and *gomezi*, averaging about 38 days from oviposition to adult emergence (table 3). Wild-caught females lay an average of 28 eggs, closely approximating the number laid by *sanguinarius*, *gomezi* and *ylephiletor* (table 2).

The eggs are long, slim, black and thick-shelled, with an adhesive substance on the shell. The larvae at all stages have very long caudal bristles. After the first instar the larvae are dark gray in color and flattened dorsoventrally. In the first two instars the head is dark but in the third and fourth there is a striking change in gross appearance. The head becomes much lighter than the body and the greatly enlarged conical bases of the antennae suggest the dorsal profile of a cat's head.

Almost all larvae while on leaves in a culture constantly manipulate the mandibles, apparently scraping the leaf surface and presumably ingesting fungi or other organic material. They also consume visible fungus growth. As a rule, larvae pupate high on the sides of the pot. In an aquarium culture which had a top layer of large leaves, the pupae were all on the upper leaf surfaces. The pupae are slender and very dark.

The larval mortality in cultures is high. Although a pot started with 200 or 300 eggs may yield 30 to 50 adults, the reluctance of *panamensis* to feed on any animal in the laboratory has prevented us from carrying this species beyond a second laboratory generation.

P. pessoana Barr.—This species is the least common of the six regularly taken in biting collections from man and horse. It is a close relative of *panamensis* and like this species is a litter- and leaf-dwelling species in nature. Possibly because conditions in the laboratory are not optimal, females lay an average of only 20 eggs, with 55 the highest number (table 2). In the laboratory the time from oviposition to adult emergence, based on incomplete and limited data, varies from 32 to 38 days (table 3), a period comparable to that of *gomezi*.

Eggs and larvae are morphologically similar to those of *panamensis* and larval behavior in cultures is the same.

Cultures of *pessoana* in rearing pots have been on the whole unsuccessful. This species is quite irregular in its distribution and in its seasonal occurrence. In one patch of dense forest, one of the few places where Mr. Hanson found both larvae and adults in dead leaves, with adults also on the green leaves of low vegetation, the temperature under the leaves (25.0° C.) was at least a degree lower than in another patch of more open woodland where the related *panamensis* was abundant and *pessoana* rare. Because of this temperature difference in nature we tried keeping pots of *pessoana* larvae in an air-conditioned room slightly cooler than the rearing room, but with no better results. However, a culture in a large aquarium kept in the cooler room produced a good number of adults. Larvae were found mainly on the upper leaf surfaces although some crawled onto the glass sides and a few were found on leaves below the upper layer but not where the leaves were closely appressed. Pupation occurred on the upper leaf surfaces and on the lower parts of the glass walls.

P. ylephiletor F. & H.—This close relative of *trapidoi* is one of the litter- and leaf-dwelling species (although unique among our principal man-biters in that the adults may also be found resting in buttresses). The time from oviposition to adult emergence averages about 40 days, with second- and third-generation cultures taking slightly longer (table 3). Wild-caught females lay an average of 27 eggs (table 2), comparable to that for *sanguinarius*.

The eggs of *ylephiletor* are slim, oval, and black, coated with an adhesive substance. Larvae have long caudal bristles, have black heads and pale bodies, are slow-moving and occur only on the surface of the food. If a particularly attractive colony of fungus is present, the larvae may remain more or less stationary for 24 hours or more, and by swinging the head, eat a semicircular hole in the mold colony. With a sudden increase in light the larvae flick the caudal bristles but do not make other movements. Pupation usually occurs on the wall of the rearing pot. In aquarium cultures the pupae are usually found on the upper side of the leaves, chiefly of the top layer but also on those beneath if the leaves are not closely appressed. Mortality in cultures is high and females are reluctant to feed in the laboratory, with the result that our attempts to produce colonies adapted to the laboratory have so far been unsuccessful beyond the third generation because of the progressive attrition in numbers.

P. trapidoi F. & H.—This species, like *ylephiletor*,

panamensis and *pessoana*, is a litter- and leaf-dweller in nature. Unlike the other common man-biters we have reared (except *pessoana*), *trapidoi* lays in the laboratory an average of only 21 eggs, although the extremes, 1 to 75, are comparable to those of the other species (table 2). The time from oviposition to adult emergence averages about 43 days in the first laboratory generation and 46 in the second (table 3).

In morphology of the egg and larva, and in larval habits in the rearing pots and in aquarium culture, *trapidoi* is similar to its close relative, *ylephiletor*, but it is definitely a more difficult species to work with in the laboratory. We have not succeeded in producing more than two consecutive laboratory generations, since larval mortality is high and females are very reluctant to take a blood meal.

SPECIES OTHER THAN THE PRINCIPAL MAN-BITERS

This group includes a number of species characteristically found in buttresses, hollow trees and animal burrows, as well as in various other habitats. Some of these species are known to bite man, e.g., *shannoni*, *cruciatus*, although the natural hosts of most of them are unknown; some are relatively rare.

A number of the failures in rearing occurred before certain of the difficulties with regard to food and molds were overcome. Further, in a number of cases the progeny of individual females were reared in the original plaster-lined vials in which the eggs were laid. While adequate in general, these vials do not provide quite so favorable an environment for the larvae as the rearing pots.

The number of days per stage and total time from oviposition to adult emergence for the following species are shown in table 5.

P. aclydiferus F. & H.—Number of eggs laid by an individual female: 69. Total days, oviposition to adult emergence: 33 (one observation).

P. apicalis F. & A.—Number of eggs laid by individual females: 10, 12, 12, 24, 26. Total days, oviposition to adult: 36 (one observation).

The larva has very long caudal bristles, is slow-moving and remains on the surface of the food. The larva is deeply pigmented (dark gray) except for the head, prothorax and the last abdominal segment. The pupa is long and slim, resembling *panamensis* in shape, and is pale centrally and dark laterally.

P. arborealis F. & A.—Number of eggs laid by individual females: 2, 9, 47, 50. Total days, oviposition to adult: 38, 40, 40, 44. The larvae occur both beneath and on the surface of the food.

P. barrettoi Mang.—Number of eggs laid by individual females: 7, 8, 53. None were carried beyond the first instar.

P. camposi Rodr.—Number of eggs laid by individual females: 12, 15 (95 by two females). Total days, oviposition to adult: 42 (one observation). The eggs are small and pale. The larvae have short caudal bristles and occur only on the surface of the food.

P. carpenteri F. & H.—Number of eggs laid by individual females: ca.12, ca.27, 56, 62, 71, 75, 77. None were carried beyond the first instar.

P. cayennensis F. & A.—Number of eggs laid by individual females: >53, 95, 95. Total days, oviposition to adult: 26, 27. The larvae have short caudal bristles, are extremely active and crawl both under and on the surface of the food.

P. cruciatus Coq.—Number of eggs laid by an individual female: 41. Total days, oviposition to adult, >45.

P. dysponetus F. & H.—Number of eggs laid by an individual female: 75. The eggs did not hatch.

P. galindoi F. & H.—Number of eggs laid by individual females: 37, 44, 50, 59, 63, 98, 113, 127. Total days, oviposition to adult: 76 (one observation). The eggs are large, oval and dark. The larva has but one pair of rather short caudal bristles during the entire cycle (a characteristic of the *Brumptomyia* group). Larvae are slow-moving and tend to hide under masses of food.

P. geniculatus Mang.—Number of eggs laid by individual females: 3, 4, 5, 6, 9, 12, 19, 34. None were carried through from egg to adult. In the one successful culture the only larva reaching the fourth instar was preserved for study. The eggs are elongate and black. The larvae are slow-moving and occur on the surface of the food. The caudal bristles are long and the larvae are dark in hue, resembling the closely related *panamensis*. One egg hatched 36 days after oviposition and two other eggs hatched after more than 36 days.

P. hamatus F. & H.—Number of eggs laid by an individual female: 80. Total days, oviposition to adult: >39. Like other members of the *Brumptomyia* group, there is only a single pair of caudal bristles throughout larval development.

While *hamatus* is rather rare in adult collections, Hanson (1961) found it by far the most abundant species recovered as larvae in nature. Out of a total of 2,258 immature stages, he reared 600 to the adult stage and of the 15 species thus identified (see table 1) 437 were *hamatus*. Nearly all of the latter were from the top inch or two of the soil between buttresses.

P. hartmanni F. & H.—Number of eggs laid by individual females: 12, 27. Total days, oviposition to adult: 53 (one observation). The egg is oval and black. The larva has short caudal bristles, a dark head and pale body. Larvae occur both below and on the surface of the food and are slow-moving.

P. isovespertilionis F. & H.—Number of eggs laid by individual females: 6 to 44; average for 20 females: 28. Total days, oviposition to adult; 36 to 45; average for 17 females: 41. The eggs are large, oval and dark. Larvae have black heads, occur below and on the surface of the food, and the caudal bristles are rather long. See further comment under *vespertilionis*.

P. nordestinus Mang.—Number of eggs laid by an individual female: 59 (one observation). Total days, oviposition to adult: 38.

This single culture is the one referred to in Part I (Hertig and Johnson 1961) which provided our

first demonstration of the potential value of leaf litter as an ingredient of the larval food. The newly hatched larvae were crawling away from the feces-blood mixture, climbing the bare walls of the pot, and would certainly have perished. Within a matter of minutes after adding a small pile of leaf litter some larvae had

dividual females: 6, 14, 15, 15, 28. Total days, oviposition to adult: 42, 42, 44, 53. The eggs are oval, dark and thin shelled. The caudal bristles are not particularly long and the larvae occur both beneath and on the surface of the food.

P. pinealis F. & A.—Number of eggs laid by an

Table 5.—Shortest number of days per stage, species other than principal man-biting sandflies.[a]

Species	Blood meal to egg	Egg	Larval instars				Pupa
			I	II	III	IV	
apicalis	8	10-11	5-6	3	8	preserved	
"	7	11	5	2	preserved		
"	7	11	4	6	4	preserved	
"	8	9	5	3	4	10	5
"	>2(gravid)	9	6	3	5	preserved	
arborealis	>4(gravid)	9	6	5	4	9	7
"	>3(")	11	6	2	5	8	8
"	>2(")	9	6	3	3	10	7
"	>3(")	9	6	5	3	14	7
camposi	>5(gravid)	10	5	3	5	preserved	
"	>4(")	8	5				11
cayennensis	>1(gravid)			2	2	preserved	
"	6	3	2	3	4	8	
galindoi	>2 to >6(gravid)	17	9	9	12	17	12
"	>3 to >6(")	13	9	9	9	preserved	
"	5	16	12	7	preserved		
geniculatus	4	16	7	5	5	preserved	
hartmanni	6	12	8	5	8	12	preserved
"	>5(gravid)	12	6	5	5	14	11
isovespertilionis	(gravid)	9-12	4-6	3-4	4-5	6-10	9-11
(17 cultures)		av:10.9	av:5.0	av:3.5	av:4.3	av:7.4	av:10.0
ovallesi	5	10	6	4	4	9	9
"	>4(gravid)	7	7	4	4	8	10
"	9	9	5	5	5	9	9
"	4					11	13
rubidulus	>4(gravid)	9	6	4	5	9	preserved
"	>6(")	12	6	4	4	preserved	
runoides	>4(")	15	8	6	8	preserved	
serranus	4	9	5	3	4	7	9
"	7	7	6	3	4	7	preserved
"	7	6	5	3	4	6	11
" (2nd lab. gen.)	6	7	5	3	4	7	7
shannoni	4	11	5	3			8
"	>4(gravid)		7	4			
"	>2 to >4(")	11	5	5	5	10	8
"	6	14	7	4	4	8	preserved
"	>5(gravid)	12	7	9	6	9	8
"	3			8	3	7	10
spinosus			7	7	6	12	10
trinidadensis	>3(gravid)	8	4	4	2	7	13
"	>4(")	10	4	3	3	8	10
triramulus	>2(gravid)	8	6	3	7	8	14
"	>6(")	8	6	4	6	7	11
vespertilionis	>0(gravid)	15	4	4	4	7	11
vexillarius	4	8	8	7	7	16	9

[a] Except as noted, each line represents a single culture, the progeny of one individual or a pool of wild-caught females.

reversed direction and soon there were a number at the leaf debris. It appeared that the mere presence of this material provided an attractive environment since the larvae seemed not to eat it but took the food they had at first refused. Later they moved about actively beneath the food and web of fungi, pushing the material aside to form tunnels large enough to accommodate the nearly erect caudal bristles. The original 59 eggs produced 56 adults.

P. ovallesi Ortiz.—Number of eggs laid by individual female: 26. The larvae were not carried beyond the first instar.

P. rubidulus F. & H.—Number of eggs laid by individual females: 2, 5, 6. Of the two successful rearings, the fourth-instar larvae or the pupae were all preserved. The larvae remain on the surface of the food.

P. runoides F. & H.—Number of eggs laid by an individual female: 19. The two larvae which survived to the third and fourth instar were preserved.

The eggs are large, oval and dark. The larva has long caudal bristles and remains on the surface of the food.

P. serranus D. & A.—Number of eggs laid by individual females: 10, 14, 33. Total days, oviposition to adult: 35, 37 (two observations).

The eggs are oval and dark. The larvae are vigorous and active, occurring both on the surface and beneath the food. This seems to be a "hardy" species; 6 ♂♂ and 2 ♀♀ accidentally left in the refrigerator for 16 hours at 4.5° C. revived and remained alive more than 24 hours after removal to room temperature.

P. shannoni Dyar.—Number of eggs laid by individual females: 5 to 92; average for 17 females: 34. Total days, oviposition to adult: 42, 44, 51 (three observations). The larva is pale in hue and has very long caudal bristles. Larvae occur only on the surface of the food.

P. spinosus F. & A.—Number of eggs laid by an individual female: 31. Total days, oviposition to adult: 52±3 days (one observation). The larvae are in constant but slow movement and are always found on the surface of the food. The caudal bristles, about as long as the body, are held erect.

P. trinidadensis Newst.—Number of eggs laid by individual females: 39 to 100; average for 12 females: 73. Total days, oviposition to adult: 38 (two observations). The eggs are large and dark. Larvae occur both beneath and on the surface of the food. The caudal bristles are not especially long. This species is one of the most abundant buttress-inhabiting species and is known to feed on geckos in nature.

P. triramulus F. & H.—Number of eggs laid by an individual female: 100 (194 by 2 females). Total days, oviposition to adult: 41, 46.

The eggs are light in hue with thin shells and the larvae are pale with light brown heads and short caudal bristles. The larvae are slow-moving and occur both under the food and on the surface. The fourth-instar larva climbs as high as possible up the walls of the pot to pupate. Two females fed on a volunteer in the laboratory but the eggs they produced did not hatch. In nature *triramulus* is often found in burrows and has never been taken by us while biting either horse or man.

P. vespertilionis F. & H.—Number of eggs laid by individual females: 13 to 64; average for 21 females: 38. Total days, oviposition to adult: 45 (one observation).

The eggs and larvae are indistinguishable in appearance and behavior from those of *isovespertilionis*. The average number of eggs per female may be lower than the number given above. Since these two species are distinguishable only in the adult male, females producing eggs which did not complete development or which yielded only females could not be included in the data given above.

While both these species are commonly found in hollow trees if bats are also present, several attempts to feed laboratory-reared *vespertilionis* on bats failed.

P. vexillarius F. & H.—Number of eggs laid by individual females: 4, 10, 16. Total days, oviposition to adult: 55 (one observation). The eggs are oval and dark. The larva has a pale head and long caudal bristles. The larvae are slow-moving and occur only on the surface of the food.

Warileya rotundipennis F. & H.—Number of eggs laid by individual females: 29, 40, 66, 66, 79.

W. rotundipennis is an avid man-biter in the areas where it occurs. All the fed females involved in our rearing attempts, taken while biting man, came from an area at an altitude of 1,200 feet, where both day and night temperatures are lower than near the coast. The fed females brought to the laboratory all died without ovipositing. It was found that if the females were kept in their normal environment, in a rearing pot partially buried in the earth, they would live long enough to produce eggs. Of the seven females which laid eggs, five took 8 days from feeding to oviposition and one laid eggs within a day, probably the result of a previous blood meal. The pots were then brought to the laboratory and until hatching were kept in either of two of the coolest available rooms at 23° or 27° C. The hatching times for those pots for which we had accurate data were 15, 15 and 16 days, a period longer than normal for *Phlebotomus* and doubtless a reflection of the lower temperature.

We were not successful in carrying the larvae beyond the first instar even though different attempts were made at room temperatures from 23° to 27° C., and the food included, in two cases, soil from the area of capture.

The egg is very small, rounded-oval in shape, and black. The first instar larva is correspondingly short and relatively much broader than *Phlebotomus*. It has very fine caudal bristles.

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Life History of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae), A Predator of the House Fly[1]

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ABSTRACT

Macrocheles muscaedomesticae is a free-living predator of the egg, and to a lesser extent of the larva, of *Musca domestica*. In the present study, emphasis is placed up standardization of general rearing conditions and a substrate (ground steer manure plus 10% soybean oil meal, moistened) as well as definition of biological performance of this mesostigmatic mite under these conditions. Eggs from virgin females were smaller (0.370 by 0.249 mm.) than eggs from mated females (0.374 by 0.256 mm.), and required a longer incubation period. These individuals spent less time as larvae; neither difference in the first two stages was significant. The protonymphal stage lasted significantly longer in the male progeny from virgin females, while the deutonymphal stage tended to be shorter. However, the total elapsed time from egg to adult for male or female progeny was not significantly different. The preoviposition period in fertilized females (53.5 hours) exceeded that in virgin females (48 hours), but not significantly. Significantly more eggs were laid by virgin females (91.7) than by fertilized females (61.4). The maximum recorded 24-hour egg production from a virgin female was 28, from a fertilized female 25; the average daily production was 7.70 and 5.29, respectively. The average life span of fertilized females (23.9 days) was close to that of unfertilized females (24.3 days); the male life span was shorter, averaging 15 days.

Macrocheles muscaedomesticae (Scopoli) Sellnick is a free-living mesostigmatic mite that is a predator of the house fly (*Musca domestica* L.) egg, and to a lesser extent the house fly larva. The first larval instar is attacked readily, but the adult mites attack the second and third instars only occasionally.

Pereira and de Castro (1945) were the first investigators to report successful rearing of *M. muscaedomesticae*. They used horse manure as a substrate and house fly eggs as food for the mites. Their paper provides excellent descriptions with measurements of the various stages of the mite, but the paper was intended to be little more than a taxonomic contribution. At that time these authors did not believe that *M. muscaedomesticae* was parthenogenetic, but in a subsequent study they found that parthenogenesis occurred and that unfertilized eggs produced only male progeny (Pereira and de Castro 1947).

The fact that the macrochelid mites are cosmopolitan is attested by the recognition which the family Macrochelidae is acquiring throughout the world. Considerable taxonomic work has been done in Great Britain by Evans and Browning (1956) and in Russia by Bregetova and Koroleva (1960). In Italy Filipponi (1955) has contributed to the general field of biology and ecology of the Macrochelidae. In this country Keegan (1959) has conducted rearing experiments with *M. muscaedomesticae*, as has Axtell (1961).

The purpose of this phase of the work was to study more critically certain areas that were left uncovered by Pereira and de Castro (1945, 1947) and Filipponi (1955) and by so doing extend the findings of these authors. Also it was felt that there was a need for standardization of rearing techniques and conditions.

METHODS AND PROCEDURE

The laboratory was maintained at 80° F. and the relative humidity ranged from 55 to 60 percent. Light was provided by fluorescent tubes controlled by a time switch to provide 15 hours of light daily. It had been previously determined, however, that the intensity or length of photoperiod had no apparent effect on the life cycle of this mite.

Stock cultures were maintained in large rectangular plastic containers 11 x 8 x 4 inches and round plastic containers 3¾ x 1¼ inches were used for smaller cultures. The large containers had organdy-covered openings on the top and the small containers had one organdy-covered opening on the top; these holes were ¾ inch in diameter. The substrate used in these containers was ground steer manure from steers fed corn silage, ground corn, and soybean oil meal as a ration. Work with the nutrition of these mites has shown that the addition of soybean oil meal to the steer manure substrate increased reproduction and generally stabilized mite develop-

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ment (Rodriguez and Wade 1961 and In press). Consequently, 10 percent soybean oil meal was used as an additive and this mixture will be referred to as the standard steer manure substrate. One gram of soybean oil meal was mixed with 9 grams of ground steer manure in the small round plastic containers and to this 20 ml. of water were added and allowed to stand until all the water was absorbed evenly. The substrate was maintained in this moistened condition. Ten young adult female mites were added to each container and after 4 to 5 days these small stock cultures contained young females of known age to conduct experiments.

Frozen house fly eggs were fed to stock cultures each day as was suggested by Keegan (1959), who provided the original culture. The freezing, of course, kills the egg and keeps the substrate free of hatching maggots. Small plastic vials, 1 inch in diameter by 2 inches, were used for individual mite rearing cells. This size of vial provided easier moisture control than smaller cells. One hole was cut in the polyethylene cover with a No. 4 cork borer and covered with organdy for ventilation. The standard rearing medium was packed down somewhat to facilitate observation of the various stages of the mites within the vial.

Mite eggs were isolated in the rearing vials and observed hourly until hatching occurred and the nymphs had molted the first time. Observations were made less frequently as the mites reached maturity. Eggs and immature stages were transferred by the use of a small needle and the adults were transferred with a No. 1 camel's-hair brush.

RESULTS

EGG.—The egg is relatively large and pearl white in color. The female makes an effort to conceal her eggs by laying them in cracks or under clumps in the substrate. At times when the eggs were removed from the cracks and placed on top of the substrate, the adult mites would stop and feed on their own eggs as if they were house fly eggs. Eggs were isolated from mated and virgin females; the eggs from virgin females are smaller than the eggs from mated females. The average dimensions of 25 eggs (mixed sexes) from mated females were 0.374 mm. in length and 0.256 mm. in width. An equal number of (male) eggs from virgin females averaged 0.370 mm. in length and 0.249 mm. in width (table 1). The eggs were isolated in the 1x2-inch vials, and they hatched in a minimum of 4.3 hours and a maximum of 14.0 hours. Incubation time differs in the two sexes but not significantly (table 2). Figure 1 shows a ventral view of a mite embryo after 4 hours of incubation.

IMMATURE STAGES.—Males and females of *M. muscaedomesticae* pass through a larval stage and two nymphal stages in their development from hatching to adult. The dimensions of the various stages and the times required for the development of all stages are given in tables 1 and 2.

Table 1.—Dimensions (in millimeters) of various stages of *Macrocheles muscaedomesticae* (25 individuals).

	Length			Width		
	Min.	Max.	Mean	Min.	Max.	Mean
Egg, from:						
Fertilized female (mixed sexes)	0.356	0.413	0.374	0.225	0.263	0.256
Virgin female (male progeny)	0.338	0.413	0.370	0.225	0.263	0.249
Larva						
Protonymph	0.38	0.45	0.42	0.26	0.30	0.27
Deutonymph	0.45	0.63	0.51	0.26	0.38	0.31
Male	0.56	0.75	0.63	0.34	0.45	0.38
Female	0.75	0.90	0.80	0.45	0.56	0.49
Adult						
Male	0.60	0.90	0.79	0.38	0.56	0.49
Female	1.01	1.13	1.08	0.68	0.79	0.74

Larva.—The duration of the six-legged larval stage ranged from 5.3 to 13.0 hours. The larval stages differ slightly in duration when they are compared as progeny arising from mated or virgin females. The male larva arising from a parthenogenetic mother takes slightly less time to pass through the larval stage (table 2). This stage evidently is not capable of feeding on house fly eggs. After the egg hatches, the larva seeks a sheltered position to await the molting into the protonymph.

Protonymph.—The average duration of the protonymph stage in females was 18.35 hours and in males from mated mothers was 15.99 hours. This stage in the males from virgin females was significantly longer than either, lasting 22.30 hours. The protonymph is opaque white in color, has eight legs, and begins to feed on house fly eggs. Adults were occasionally observed catching and feeding on nymphs in this stage.

Deutonymph.—The average duration of the male and female deutonymphs from mated mothers was 22.43 hours and 23.02 hours, respectively, while that of the male deutonymph from an unfertilized female was 19.64 hours. These differences, however, were not significant (table 2). This stage is very active

Table 2.—Duration in hours of various stages of *Macrocheles muscaedomesticae* at 80° F., using standard substrate and frozen house fly eggs.

Stage	From fertilized females		From virgin females
	Haploid male	Diploid female	Haploid male
Egg	7.33±1.10	6.89± .52	8.81±1.69
Larva	8.76±2.34	8.09±1.16	7.59±1.04
Protonymph	15.99±2.06	18.35±2.10	22.30±1.82
Deutonymph	22.43±2.50	23.02±3.04	19.64±2.22
Total hours, egg to adult (25 individuals each)	54.51±3.28	56.35±3.46	58.34±2.34

95% confidence interval.

FIG. 1.—Ventral aspect of embryo of *M. muscaedomesticae* after 5 hours of incubation at 80° F.

and feeds vigorously. As the deutonymphs develop, they may be distinguished as to sex. The male is somewhat smaller than the female and it possesses a tapered, but blunt-shaped opisthosoma. Copulation apparently occurs immediately after the last molt of the female deutonymph.

The total time required from egg deposition to molting into the adult stage averaged 54.51 hours for the male originating from the mated mother, compared to 58.34 hours for the male developing from the virgin female. The female was intermediate, taking 56.35 hours. These differences, however, were not significant (table 2).

ADULT.—Both males and females are light brown in color when first emerged from the deutonymphal stage, but darken to a dark brown in about a day. The females have an oval body which when actively feeding becomes distended, particularly the opisthosomal membrane between the ventral and dorsal plates. The ventral plates (sternal, genital, and ventri-anal shields) of the female have distinct shape, chaetotaxy, and ornamentation. *M. muscaedomesticae* has simple sternal setae. The metasternal shields are free and each carries a simple seta. The genital shield, truncate posteriorly, bears a simple pair of setae. The ventri-anal shield, approximately 368μ x 379μ, has a network of punctate lines and simple setae. It has three pairs of preanal setae in addition to three setae close to the anus (fig. 2). The dorsal shield of the female is reticulated (fig. 3). Setae are both plumose and simple, there being 28 pairs of setae and 22 pairs of pores (Evans and Browning 1956).

There is a marked change in the males from the deutonymphal to the adult stage. Two pairs of spurs on the fourth pair of legs become quite prominent and a smaller spur may be noticed on the second pair of legs (fig. 4).

The adult mites feed on the house fly egg and on the first-instar larva of the house fly. Also, under laboratory conditions they will cluster on the adult fly and suck the body fluids. Observations show that in the field the adult house fly may attract one or more mites when it is emerging from the puparium and during the teneral condition that follows. The dimensions of adult male and female mites are given in table 1.

The preoviposition period in fertilized and virgin females differs slightly but the difference is not significant (table 3). The preoviposition period for fertilized females ranged from 41 to 66 hours with a mean of 53.5 hours. The preoviposition period for virgin females was slightly less, ranging from 30.5 to 71 hours with a mean of 48 hours. The duration of the oviposition period for virgin females was 6 to 16 days, with a mean of 11.8 days. The oviposition period for the fertilized females was quite similar, being 5 to 16 days, with a mean of 11.6 days (table 3).

Egg production in virgin females ranged from a minimum of 31 to a maximum of 145. In the fertilized mite, egg production decreased significantly (table 3). The minimum number of eggs or nymphs recorded from a single fertilized female was 23 and the maximum was 109. In a 24-hour egg-laying period, the highest number of eggs for a virgin female was 28, compared to 25 from a fertilized female.

An experiment designed to study the longevity of *M. muscaedomesticae* included 37 males, 20 fertilized females, and 20 virgin females individually isolated in 7-dram vials. They were fed amply and the substrate was changed when necessary. The males were found to live an average of 15 days, but if a food shortage exists and they have to com-

Table 3.—Duration, longevity, and egg productivity of *Macrocheles muscaedomesticae* females at 80° F. using standard steer manure substrate and frozen house fly eggs (20 individuals each group).

Females	Duration of Period			
	Preoviposition (Hours)	Oviposition (Days)	Longevity (Days)	Egg productivity
Fertilized	53.5±7.6	11.6±2.2	23.9±5.4	61.4±11.2
Virgin	48.0±5.8	11.9±1.9	24.3±2.6	91.7±16.4

95% confidence interval.

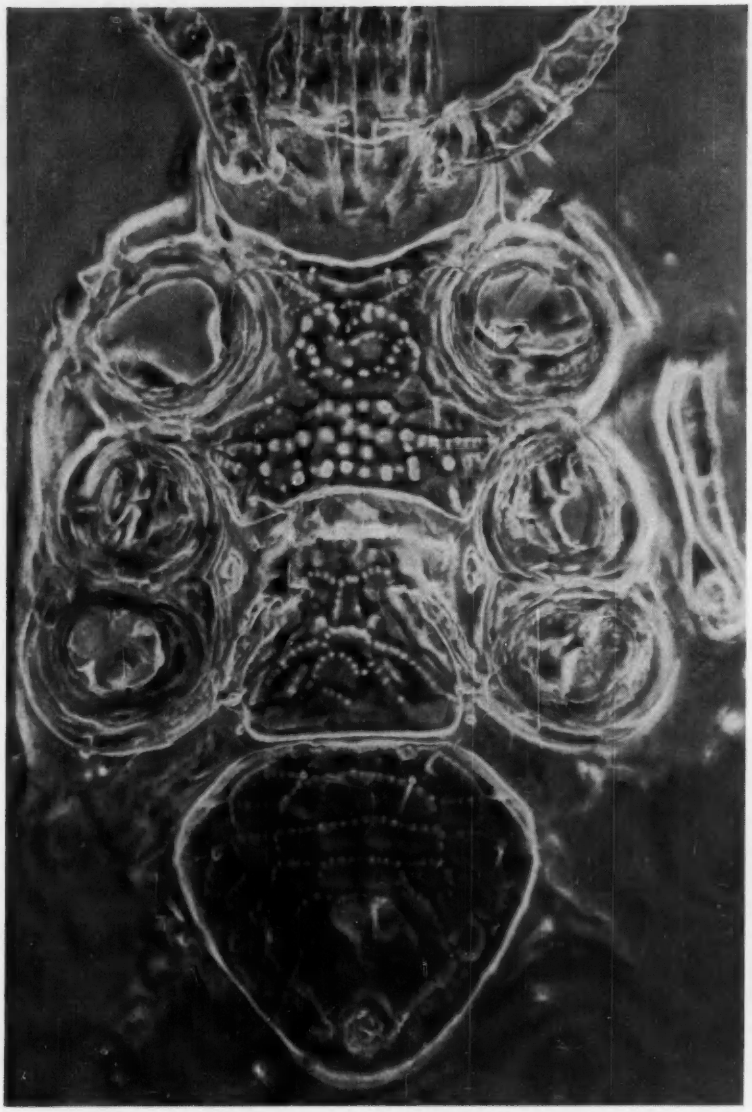

FIG. 2.—Sternal, genital, and ventri-anal shields of *M. muscaedomesticae* female, showing pattern of punctate lines.

pete with the more vigorous females, the males may die earlier than 10 days of age. Females kept in petri dishes on moistened filter paper were observed to live as many as 21 days without food at 80° F. The virgin females had a life span that ranged from 13 to 36 days, with a mean of 24.3 days, compared with a range of 11 to 58 days and a mean of 23.9 days for the fertilized females (table 3).

Parthenogenesis occurs, the haploid number of chromosomes producing male progeny. The early study by Pereira and de Castro (1945) ignored its status but later these workers (1947) proved its existence. In our studies, 21 protonymphs were isolated in 7-dram vials until mature, when their offspring were collected daily, then isolated in separate vials in the same manner as their mothers. At the end of the third day of isolation the sex of each parthenogenetic offspring was recorded. After noting 121 male deutonymphs the observations were discontinued.

In order to study sex ratio, 16 young females were taken from a stock culture containing both sexes

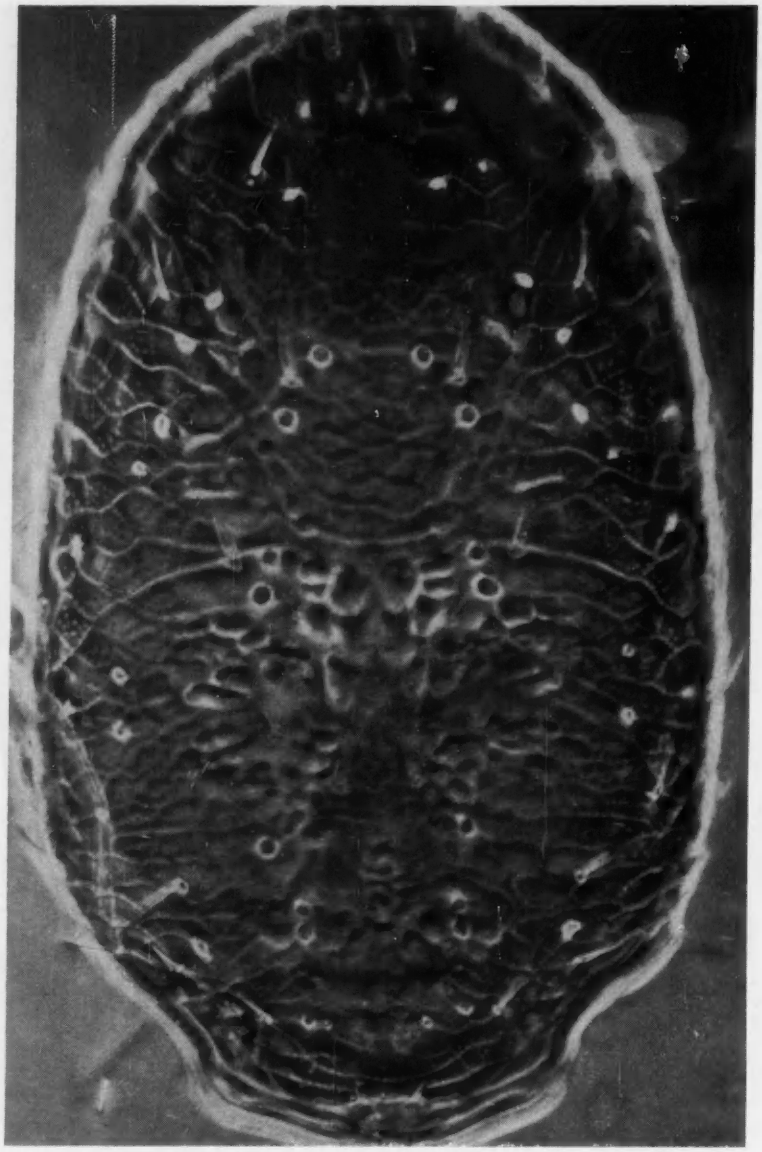

FIG. 3.—Dorsal shield of *M. muscaedomesticae* female, showing reticulation pattern and setal pattern.

and each female was isolated with a male. The sex of the offspring was recorded and of a total of 144 progeny, 56.7 percent were males and 43.3 percent were females.

DISCUSSION

It was considered important to rear *M. muscaedomesticae* under standardized conditions, since all previous reports give little information in this regard, leaving one to conclude that substrate and temperature were not standardized. Pereira and de Castro (1945) stated that the reproductive potential was about one mite per day from a given mother. Later they changed this statement to one to two progeny per day (Pereira and de Castro 1947). It has been noted that the composition of the substrate is of primary importance in defining the biotic potential of *Macrocheles muscaedomesticae* (Rod-

FIG. 4.—Adult male of *M. muscaedomesticae*, showing spurs on legs II and IV. Leg IV possesses two pairs of spurs.

riguez and Wade 1961). Hence, the reproductive rate described in the results is given only to show a comparison between fertilized and unfertilized females during their lifetime. On the basis of an average oviposition duration of 11.6 and 11.9 days for the fertilized and unfertilized females, respectively, the daily reproductive rate would average 5.29 and 7.70 eggs daily. This relatively high reproductive rate was recorded as the result of the practice of removing eggs and nymphs from the vials daily which largely prevented cannibalistic action. The habit of the macrochelids is to carry the first pair of legs raised above the body as antennae. This species uses leg I for tapping the substrate as it walks. Leg I is waved and weaved in the air constantly, and while this type of behavior is responsible for the effective predator action against the house fly egg and the first-instar maggot, it also enhances a cannibalistic action which exerts itself under some conditions. Even though the mother hides her eggs, she may later uncover and eat them, or the larvae may be attacked.

Arrhenotokous parthenogenesis occurs in a number of acarine groups. The tetranychid mites are notable examples, as are the macrochelid mites. The unfertilized (haploid) egg is smaller than the fertilized (diploid) egg and the apparent difference in dimension and volume probably is an important factor in contributing to the difference in egg production between virgin and fertilized females. That is, there is almost a 33 percent difference in egg production in favor of arrhenotokous parthenogenesis.

No explanation can be offered at this time why the protonymphal stage of males from unfertilized mothers should last significantly longer (22.30 $\pm$ 1.82 hours) than males originating from mated mothers (15.99 $\pm$ 2.06 hours). However, the overall developmental time of males (haploid) originating from unfertilized mothers, from egg to adult, was not significantly different from those originating from eggs (also haploid) of fertilized mothers.

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The Nutrition of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) in Relation to Its Predatory Action on the House Fly Egg[1]

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ABSTRACT

The effects of substrates, livestock feed additives, pH, temperature, and relative humidity were studied as factors having possible influence on the reproduction of *Macrocheles muscaedomesticae* (Scopoli). When fresh steer manure was air-dried and ground, and used as a substrate, together with 10% soybean oil meal and frozen house fly eggs as food, at 80° F. and 55 to 60% relative humidity, this species produced 4 to 5 progeny per female mite per day. Individually reared fertilized females produced up to 25 eggs per day. Used fly larval media appeared promising as a substrate for the mites. This species was found capable of ingesting semi-solids. House fly eggs were soaked in an isotonic solution of carnitine; certain levels of carnitine improved mite reproduction. Biological control tests in the laboratory showed that the amount of predation varied with the type of substrate and periods between feedings. Female mites starved for periods greater than 36 hours did not generally effect a greater predation than those starved for 24 to 36 hours. A mean of 19.8 eggs per mite was destroyed when eggs and mites were in contact for 24 hours on filter paper. Conditioned CSMA fly larval media and used fly larval media effected similar predation. When the substrate was ground steer manure, predation dropped to 8.5 eggs per mite. When fresh steer manure was used under semi-field conditions, predation was approximately 5 to 6 eggs per mite.

If it were not for the many biological control agencies at work, the house fly population would be astronomical. Each stage of the house fly is subject to attack by parasites or predators. West (1951) grouped taxonomically the natural enemies of *Musca domestica* L. and related species under the following categories: 1) fungus and bacterial infections, including rickettsiae, viruses, and spirochetes; 2) protozoan organisms; 3) roundworms and flatworms; 4) insects and other arthropods; 5) reptiles and amphibians; 6) birds; 7) insectivorous mammals.

The Arthropoda worthy of mention, according to West, are represented in the classes Chilopoda, Arachnida, and Hexapoda.

Some of the Acarina, or mites, have been noted to be associated with the house fly, *Musca domestica*. West noted such acarologists as Berlese, Banks, Ewing, and of more recent times, Pereira and de Castro, who have recorded mites taken from house flies. The mites listed include representatives from the families Trombidiidae, Tyroglyphidae, Acaridae, and the superfamily Parasitoidea.

Pereira and de Castro (1945) were of the opinion that *Macrocheles muscae* Ewing and *Holostaspis badius* Koch are synonyms of *M. muscaedomesticae* (Scopoli) and treated them as such. *M. muscaedomesticae*, according to these authors, is a rather common arthropod enemy of the house fly in Brazil. Baker et al. (1956) and Bregetova and Koroleva (1960) suggested that it is worldwide in distribution. Baker and Wharton (1952) stated that the habits of some macrochelids indicate that this group may be of considerable importance from an economic and medical standpoint. This also is the conclusion drawn from the work of Filipponi (1955, 1960) who studied the association of *M. muscaedomesticae* and the house fly, and Pereira and de Castro (1947) who studied the phoretic relationship between *M. muscaedomesticae* and the house fly adult.

Our interest in macrochelids began when they appeared in our samplings of bluegrass pasture turf plugs, where they occurred under varied situations. Concerted effort in our laboratory in the direction of investigating the possibilities of mass-rearing *M. muscaedomesticae* for biological control studies focused attention on the important role that the composition of the substrate plays on the reproductive rate and the general well-being of the mite. The present report deals with the work relative to the development of the substrates and to the biological control which may be expected from *M. muscaedomesticae* under varying conditions.

EXPERIMENTS WITH SUBSTRATE

Procedure.—The laboratory was maintained at 80° F. and the relative humidity ranged from 55 to 60 percent. The stock cultures were handled and fed as has been described (Wade and Rodriguez, 1961). The comparative tests on substrate were made using round plastic boxes 3¾ x 1¼ inches for rearing containers. Each container was ventilated with a ¾-inch hole in the center of the lid.

A total of 10 grams of substrate was used in each container in the tests, and whenever the substrate permitted, 20 ml. of water were added to each container. Each treatment was replicated four times and each experiment was usually repeated two to four times. The same treatment or variations of these treatments from previous tests were run as the tests progressed.

The maintenance of the proper moisture level was of utmost importance and this level was maintained on a daily basis. It was not possible to add a set amount of water because as the particular substrate became biologically active its water retention characteristics changed.

An incubator of the commercial chicken hatchery type was also used for some of the work.

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At the outset 10 young female mites were placed in each of the containers, and the developmental time was 10 days. It was soon ascertained that five mites developing for 5 days constituted a more profitable procedure. At the end of 5 days the rearing substrate and progeny were placed in 6-inch Nalgene funnels which were treated with Siliclad. Gentle reflected heat over the substrate made this type of extraction apparatus extremely effective in separating the mites from the substrate. They were then counted and records were kept of adult males and females and of immature forms. The results were expressed as progeny per female mite (originally introduced) per day. This method of analyzing the data made possible the comparison of various treatments over a period of time.

The materials tested as substrates varied from inorganic substances such as vermiculite and sand to materials of organic origin such as peat moss and sawdust which are not ordinarily found in the breeding sites of the house fly. Materials of a cellulose nature often created a mold problem and in the early stages of this work some effort was made to control the mold. Propionic acid was one of the better materials used for this purpose. It became evident, however, that such mold-growing materials were not conducive to good mite development, and the use of these was either avoided or their mold-growing characteristics were nullified by dilution with manure.

In a study designed to investigate the possibility of bypassing the procedure of dosing the house fly with radioisotopes to obtain radioactive eggs, it was found that it is possible to obtain a passage of P^{32}, Rb^{86}, Sr^{89}, Y^{91} and Zn^{65} into the intact house fly egg by employing an isotonic solution as a carrier (unpublished data). These results suggested employment of the same technique to introduce vitamins or vitamin-like materials into the house fly egg. One of the materials introduced was carnitine. This was done by soaking the eggs in an isotonic solution containing carnitine for several hours before refrigerating. The eggs remained in the solution from 24 to 48 hours before being fed to the mites.

RESULTS

Table 1 lists a number of substrates which range in pH from 4 to 9. It was concluded that pH near 7.0 was the most desirable and that the lowering of pH to 4 to eliminate molds was detrimental to the mites, as that treatment significantly decreased mite reproduction when compared with a number of substrates in the alkaline range. It was also shown that an organic base substrate is necessary, as vermiculite did not support mite reproduction. The age and type of manure are of the utmost importance, as fresh manure originating from steers on a fattening ration was superior as a substrate to old manure obtained from dairy cows. The remaining manure treatments were from beef animals but were not necessarily fresh manure treatments and none of the manure substrates in this series were ground. They were, however, dried and broken into small fragments.

Table 1.—The effect of pH and substrate on reproduction of *Macrocheles muscaedomesticae* at 80° F. when fed frozen house fly eggs.

Substrate	ph	Progeny per female per day[a]
Vermiculite + HOH	7	.18±.04
Vermiculite + CaOH + HOH	9	.10±0.8
Vermiculite + manure extract	7	.45±.16
Manure + wood shavings (1:1) + 0.2% propionic acid	4	.28±.20
Manure + wood shavings (1:1) + 0.2% propionic acid + NaOH	8	1.48±.50
Manure + wood shavings (1:1) + ammonium nitrate	8	.40±.24
Manure + wood shavings (1:1) + CaOh	9	1.47±.56
Manure + wood shavings (1:) + manure extract	7	1.46±.38
Manure (fresh steer) + wood shavings (1:1) + HOH	7	1.92±.74
Manure (old dairy) + wood shavings (1:1) + HOH	7	.50±.11

[a] 95% confidence interval.

The superiority of fresh steer manure over old dairy manure led to the testing of livestock feed additives as shown in table 2. These tests resulted in the adoption of steer manure plus 5% fish meal as a standard, which produced almost 2 progeny per female per day. Several bell-shaped relationships resulted upon the addition of some additives to the

Table 2.—The effects of livestock feed additives in three concentrations on reproduction of *Macrocheles muscaedomesticae* at 80° F. when fed frozen house fly eggs.

Substrate	Progeny per female per day[a]		
	2.5%	5.0%	10.0%
Substrate standard plus:			
Aureomycin[b]	1.10±.63	1.65±.27	1.18±.11
Rolled oats[c]	.50±.18	.66±.05	.47±.26
Fish meal, 60% protein[d]	1.49±.81	1.99±.75	.77±.56
Pfizer vitamin supplement[e]	1.05±.38	1.38±.46	1.48±.13
Pfizer vitamin B_{12} supplement[e]	.74±.33	.66±.36	.84±.42
Distillers dried solubles, 25% protein[f]	.82±.27	.76±.25	.92±.51
Super sol (distillers solubles + vitamins)[f]	.70±.23	.87±.24	.60±.11
Powdered skim milk (Starlac)[g]	.75±29	1.53±.83	.99±.68
Substrate standard (manure + wood shavings 1:1)		.67±.10	

[a] 95% confidence interval.
[b] American Cyanamid Company.
[c] The Quaker Oats Company.
[d] Aubrey Feed Mill.
[e] Charles Pfizer & Company.
[f] Brown-Forman Distillers Corporation.
[g] The Borden Company.

substrate standard at 2.5, 5.0 and 10.0%. These were aureomycin, fish meal, and powdered skim milk. These relationships indicated optimal levels of additives. Rolled oats produced a considerable amount of mold which impeded mite development. The substrate standard was of the same nature as that utilized in table 1.

The series given in table 3 included additives with various types of manure. At this particular point in the development of substrate, the value of grinding the manure was recognized, and this procedure was adopted routinely. Fresh steer manure was air-dried and then ground in a feed mill. The addition of 10% soybean oil meal to ground steer manure resulted in the best reproduction obtained thus far (3.50 progeny per female per day), and this became the new standard substrate.

Table 3.—The effects of various manures and feed additives on reproduction of *Macrocheles muscaedomesticae* at 80° F. when fed frozen house fly eggs.

Substrate	Progeny per female per day[a]
Grass-fed cow manure	1.23± .16
Grass-fed cow manure + 5% fish meal	2.10±1.12
Horse manure	.91± .39
Horse manure + 5% fish meal	.94± .13
Horse manure + 10% fish meal	1.10± .18
Horse manure + 5% soybean oil meal, 44% protein	1.33± .47
Horse manure + 10% soybean oil meal	1.22± .34
Ground steer manure + 5% soybean oil meal	3.11± .59
Ground steer manure + 10% soybean oil meal	3.50± .77
Ground steer manure + 15% soybean oil meal	2.49± .34
Old ground steer manure	.62± .42
Old ground steer manure + 10% soybean oil meal	.94± .50
Substrate standard (steer manure + 5% fish meal)	2.78± .26

[a] 95% confidence interval.

The series of tests shown in table 4 points out the importance of a good quality manure in the standard substrate. It was obvious that the quality of this particular batch was not equal to that used in some previous standard substrates. Increased humidity improved mite reproduction slightly. The tests run at 43% and 53% relative humidity and 88.5° F. were conducted in the incubator and were compared to laboratory conditions recorded at 48% R.H. and 80° F. The incubator again was employed to produce a 68% R.H. at 88.5° F. in order to compare the series shown with a duplicate series in the laboratory (table 5). The standard substrate produced approximately the same number of progeny in both sets of conditions. Liver powder at 48% and 80° F. effected a significant increase in progeny over the standard and also over liver powder at 68% and 88.5°, indicating that the same substrate may react differently at different conditions of temperature and humidity. Some treatments were significantly lower than the standard at one or both levels of temperature and humidity.

Table 4.—The effects of substrates, additives, temperature (F.) and relative humidity on reproduction of *Macrocheles muscaedomesticae* when fed frozen house fly eggs.

Treatment	Progeny per female per day[a]		
	R.H. 43% Temp. 88.5°	R.H. 48% Temp. 80°	R.H. 53% Temp. 88.5°
Standard plus:			
Wood chips, 5%	.84 + .11	1.46 + .38	———
Dried blood, 10%	.63 + .09	.98 + .33	———
Liver extract, 10%[b]	.84 + .11	1.02 + .52	———
Liver powder, 10%[b]	.62 + .14	.58 + .17	———
Soy hydrolysate, 10%[b]	———	.39 + .13	.37 + .11
Alpha protein, 10%[b]	———	1.22 + .22	.79 + .14
Alpha cel, 10%[b]	———	1.36 + .28	1.71 + .58
Liver extract, 10% + alpha protein, 10%	———	1.12 + .15	.80 + .20
Standard (steer manure + soybean oil meal, 10%)	1.55 + .30	1.59 + .20	1.67 + .60

[a] 95% confidence interval.
[b] Nutritional Biochemicals Corp.

It was deemed important to define the exact influence of substrate and food (house fly eggs). Used fly larval media suggested itself as a substrate. After the fly larvae were reared the material was air-dried and ground. Fly larvae were not reared in the "new" fly larval media tested; this was utilized after 3 to 4 days of fermentation (this conditioned the substrate and eliminated mold formation) after having a processing similar to that given the used larval media. When house fly eggs were omitted from the fly larval media, virtually no reproduction occurred. Steer manure as a substrate is superior to fly larval media, and a small increase in reproduction was observed even without fly eggs.

As feed additives were introduced to the new and used fly larval media, increases in reproduction were

Table 5.—The effects of substrates, additives, temperature (F.) and relative humidity on reproduction of *Macrocheles muscaedomesticae* when fed house fly eggs.

Treatment	Progeny per female per day[a]			
	R.H. 48%	Temp. 80°	R.H. 68%	Temp. 88.5°
Fresh fly eggs plus:				
Fly larval media, used[b]	2.40 ±	1.32	3.82 ±	1.49
Fly larval media, new	2.13 ±	.17	1.41 ±	.73
Frozen fly eggs plus:				
Fly media, used + soybean oil meal, 10%	1.56 ±	.64	1.71 ±	.45
Fly larval media, new + soybean oil meal, 10%	2.32 ±	.94	.79 ±	.63
Standard and frozen fly eggs plus:				
Liver powder, 5%	4.35 ±	.56	2.94 ±	.57
Liver extract, 5%	1.94 ±	.30	2.41 ±	.42
Dried blood, 10%	1.10 ±	.55	.91 ±	.36
Soy hydrolysate, 10%	2.88 ±	1.37	2.50 ±	.90
Standard (steer manure + soybean oil meal, 10%)	2.59 ±	.32	2.70 ±	.48

[a] 95% confidence interval.
[b] Standard Chemical Specialties Manufacturers Association (CSMA) medium; Ralston-Purina Co.

marked, the greatest progeny return being from used fly larval media plus 5% fish meal plus 10% soybean oil meal, which gave 2.6 progeny per female per day. The performance of used fly larval media indicated that there is some entity of some stage of the fly left which is beneficial to mite development. (Table 6.)

Table 6.—The effect of ground fly larval media, and additives on reproduction of *Macrocheles muscaedomesticae* at 80° F. with relative humidity of 58%.[a]

Treatment	Progeny per female per day[b]
Fly larval media, used, no fly eggs	.06± .07
Fly larval media, used	1.11± .28
Fly larval media, new, no fly eggs	.11± .04
Fly larval media, new	1.08± .47
Steer manure, no fly eggs	.41± .13
Steer manure	2.04±1.46
Fly larval media, used, + fish meal, 5%	.69± .52
Fly larval media, new, + fish meal, 5%	.84± .28
Fly larval media, used, + soybean oil meal, 10%	1.39± .36
	.63± .22
Fly larval media, new, + fish meal, 5% + SBOM[c], 10%	.96± .34
Fly larval media, used, + fish meal, 5% SBOM, 10%	2.60±1.38
Fly larval media, new, + fish meal, 5% + SBOM, 10%	.64± .13
Standard (steer manure + soybean oil meal, 10%)	4.15± .82

[a] House fly eggs added unless otherwise specified.
[b] 95% confidence interval.
[c] Soybean oil meal.

This particular series of substrates pointed out the necessity for an interpretation of whether the mite actually ingests substrates, as had been suspected all along. Soybean oil meal was dyed with safranin and then dried, and mites were introduced to this substrate with no moisture. After a day in this material, newly molted adults were fixed on a drop of immersion oil and photographed, as were mites that had been fed on house fly eggs dyed with safranin. Mites that had merely fed on house fly eggs on filter paper were used as controls. It was unmistakably demonstrated that these predator mites are capable of ingesting semisolid materials such as soybean oil meal (figs. 1 and 2). Dissections with a micromanipulator confirmed this.

In the experiments directed at introducing carnitine into the house fly egg by the use of an isotonic solution as a carrier for DL carnitine hydrochloride, the carnitine levels at 0.025 and 0.05 mg. per ml. significantly improved reproduction in this mite when compared to the untreated standard steer-manure substrate (fig. 3).

BIOLOGICAL CONTROL STUDIES

Procedure.—Laboratory tests and tests under semi-field conditions were conducted in order to study the potential of *M. muscaedomesticae* as a predator of the house fly. Again test conditions were standardized as much as possible in regard to micro and

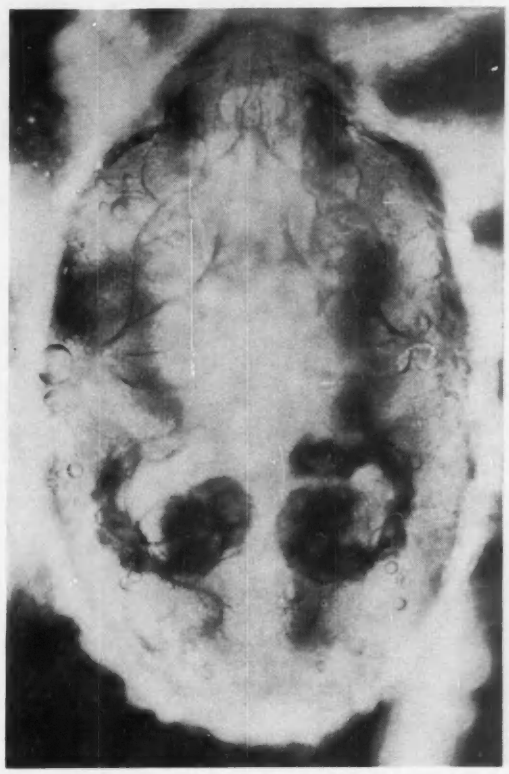

FIG. 1.—Newly molted *M. muscaedomesticae* female adult fed on safranin-dyed house fly eggs, showing (dyed) opaque gut.

macro environment. The house fly eggs that were introduced were less than 3 hours old and the mites were young female adults that had just begun to oviposit but of known condition in regard to the last feeding. This state was achieved by feeding the mites, removing them from the organic substrate and placing them in a container providing damp vermiculite for a substrate until they were removed once more to begin the biological control test.

The simplest test employed the use of the 3¾ x 1¼ inch rearing container already described in which a damp filter paper was used instead of substrate and to provide humidity. A known number of house fly eggs were introduced (250 per replicate) followed by five young female mites per container. A contact time of the mite and egg (or maggot) of 24 hours was provided for this type of test. For tests employing fly larval media it was found that mold interfered with mite predatory action and limited data were obtained with the use of gallon battery jars (the container ordinarily used to rear the house fly larvae in our laboratory). The particular series given in table 7 were run concurrently. In the filter paper series the mite-egg (or mite- developing first instar maggot) contact was for 24 hours.

FIG. 2.—Newly molted *M. muscaedomesticae* female adult fed on safranin-dyed soybean oil meal, showing (dyed) opaque gut.

The deflated egg cases were counted as were mites and progeny. The remaining substrates were inoculated with house fly eggs and the mite contact with egg or maggot was 60 hours, at the end of which time the mites and fly larvae were counted. As was discussed in the previous substrate section, however, ground, used, fly-larval media and ground, new, fly-larval media, which had been fermented and conditioned, provided a substrate which did not mold and which also gave varied comparison with the standard ground steer manure substrate.

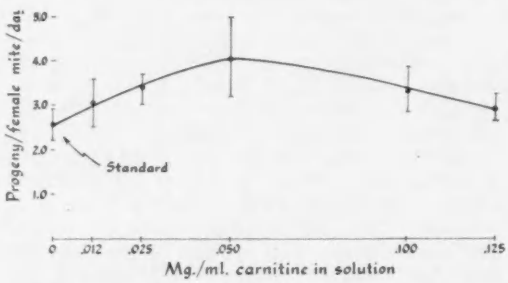

FIG. 3.—The effect of carnitine on the reproduction rate of *M. muscaedomesticae*. An isotonic solution was used as a carrier for DL carnitine hydrochloride, and house fly eggs were soaked in it before being fed to mites.

Fresh steer manure (a few minutes old) was placed in galvanized boxes, 6 x 6 x 12 inches, open at both ends and then screen-capped at the top. House fly eggs were introduced and then buried slightly, as were mites. The boxes were placed at a manure pile and also in a greenhouse during the summers of 1959 and 1960. In the fresh manure testing, moulting mites and maggots were counted after 72 hours' exposure of egg or maggot to mite.

RESULTS

Table 7 summarizes a typical laboratory experiment. There are unpublished data that indicate that starving mites more than 24 hours does not produce more predatory action and this summary (table 7) substantiates those data. Indeed, there were indications that starving the mites 60 hours might actually result in lessened predatory action. The more noteworthy results, however, are the differences shown between types of substrates. Predatory action on the filter paper obtained a range of control in keeping with previously obtained results and approximately 15 eggs per mite would represent a mean. Steer manure as a substrate provided the lowest biological control. The fly larval media, both new and used, enhanced predatory action, when compared to the standard steer manure substrate.

Table 7.—A typical biological control test series in the laboratory (56% R.H. and 80° F.) showing the effect of starvation and type of substrate on *Macrocheles muscaedomesticae*.[a]

Substrate	Mites last fed (hrs.)	Live fly larvae count	Fly larvae not hatched	Fly larvae not hatched due to predation	
				Total	Mean per mite[b]
Filter paper	36	167	833	395	19.8± .7
	60	131	869	221	11.1±1.0
Steer manure + 10% SBOM[c]	36	391	609	169	8.5±3.0
	60	143	857	167	8.4±2.4
Fly larval media, new	36	207	793	443	22.2± .8
	60	290	710	296	14.8±2.2
Fly larval media, used	36	94	906	346	17.3±1.1
	60	289	711	682	34.1±1.6

[a] A total of 20 young females and 1,000 house fly eggs (4 replicates) were used per treatment. Mites were exposed to egg or developing maggot for 24 hours on filter paper, 60 hours on other substrates.
[b] 95% confidence interval.
[c] Soybean oil meal.

In limited tests using battery jars and CSMA fly larval media predatory action was relatively low, and an average of only 5.1 eggs or maggots per mite were controlled. Mold interfered with the predatory action of these mites, however, and it was noticed

that a condensate formed on the walls of battery jars due to the fermentation process. This also interfered with the movement of the mites. In the limited semi-field tests which were conducted in the field and in the greenhouse one of the problems encountered was the low hatchability of the house fly egg in the fresh steer manure. Approximately 30 percent was the average hatch, and the percent control under those conditions compared favorably with the battery jar tests (Rodriguez and Wade, in press).

DISCUSSION

In achieving a relatively high reproductive rate of from 4 to 5 progeny per female per day in nutritional tests, the question of reproductive potential is paramount, and reference to life history and biological studies is relevant (Wade and Rodriguez 1961). It was found that fertilized female mites produced an average of 5.29 eggs per female per day over an ovipositional period of 11.6 $\pm$ 2.2 days. The egg production for virgin females was 7.70 eggs per female per day. Such results were obtained by removing larvae and protonymphs from the individual rearing vial every few hours. This procedure was followed to lessen the probability of cannibalism which evidently occurs when food or substrate is inadequate or population too dense or restricted in the rearing container.

It would appear, therefore, that four to five progeny per female per day are a relatively effective reproductive return, as has been achieved under our experimental conditions. This is not to say that we have obtained all of the reproductive potential from this species, because, as has been observed, individual fertilized *M. muscaedomesticae* females have been noted to produce as many as 25 eggs per day each, at peak production. Nor have we fully investigated the extent of predation manifested by other macrochelids. Studies are now in progress in this area.

The macrochelids have a primitive type of mouthparts and their chelae are opposable, toothed, and well adapted for biting or crushing. It was no surprise, therefore, to find that *M. muscaedomesticae* are capable of ingesting what would appear to be solid material, as soybean oil meal. It is still a possibility, furthermore, that certain critical nutritive requirements of the mite may be satisfied by absorption through the soft integument in the larval and nymphal stages. It remains to be seen how much of a role liquids or semiliquids play in its diet.

The role that moisture plays in the general well-being of this mite is observable when substrate or stock cultures go dry. Reproduction ceases and the larvae and nymphs die first. In the work reported, the microenvironment in the container was damp all the time. Grinding the substrate undoubtedly assists in stabilizing moisture, as well as making more nutrients available. The relative humidity of macroenvironment simply determined the rate of water vapor exchange between the micro- and macroenvironment. The data indicate that relative humidity in the macroenvironment in the 55 to 65 percent range improves reproduction over the 40 to 50 percent range. When temperature was increased from 80° to 88.5° F. (and humidity increased from 48 to 68 percent), dried blood and liver powder as additives to the standard substrate reacted detrimentally to reproduction rate. Ammonia was liberated and apparently the concentration was too high for good mite development.

In attacking the house fly egg, *M. muscaedomesticae* bites through the chorion and the contents are seemingly sucked. Feeding on the first instar maggot appears to be in the nature of crushing tissue; as the maggot develops, its integument apparently becomes too leathery for the mite to attack. There is no question that the mites' first preference is for house fly eggs; when these are introduced, within minutes the mites attack them hungrily. They appear to have no difficulty in finding eggs that have been buried in the substrate. This is demonstrated by the fact that on the fly larval media, the biological control obtained was higher than that on damp filter paper where the eggs were readily available. When ground steer manure was used as a substrate in biological tests in the laboratory, predation dropped to 8.5 eggs per mite, or less than one-half that obtained when eggs were exposed to mites for 24 hours on filter paper. The laboratory results substantiated findings made under semi-field conditions. Perhaps less predation occurs with manure substrates because other nutrients seem to be available to the mite from the manure. This is particularly true if the manure is relatively fresh. Another distinct possibility is that the other substrates, such as fermented fly larval media are giving off gases or substances which irritate the mites, causing a speedup in metabolism (not evidenced in reproduction rate) and causing the mites to be restless and to rupture more house fly eggs than they are capable of consuming.

The role of microorganisms has not been studied. However, when the livestock feed additive aureomycin was introduced into the substrate, the indications of beneficial and detrimental effects appeared, depending on the level of aureomycin used.

Likewise, vitamins need to be investigated thoroughly, towards our eventual goal of developing a standardized diet. The carnitine study showed that it was beneficial to mite development at certain levels when introduced in the house fly egg. This is noteworthy because of the chemical relationship between carnitine and choline, the latter being found in manure. Carnitine has been shown to be essential in the nutrition of tenebrionid beetles, and it can replace choline in the diet of *Drosophila* (Lipke and Fraenkel 1956).

While phoresy exists between this mite and the house fly, it has been observed that flies in the teneral condition are sometimes attacked by a cluster of mites and the adult is unable to fly. When this happens to flies in captivity, the mites soon kill the fly.

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Some Histological Aspects of the Boll Weevil[1]

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ABSTRACT

Histological studies of microtome sections of fixed specimens of the boll weevil (*Anthonomus grandis* Boh.) revealed the general structure of the alimentary canal, stomodaeum, mesenteron, proctodaeum, Malpighian tubules, male reproductive organs, fat bodies, oenocytes, labial glands, muscle and attachment to body wall. Detailed drawings showing these structures are given.

In the course of studies to determine histopathological changes that may occur in the boll weevil (*Anthonomous grandis* Boh.) exposed to insecticides, it became evident that little information was available on the histology of the Rhynchophora in general and none on that of the boll weevil. With the thought that even limited histological studies might contribute to the overall knowledge of the histology of insects, the following observations are recorded. An exhaustive study of the histology of this insect was not attempted; rather, the observations were limited to the more general aspects of microtome sections cut from fixed specimens embedded in any suitable embedding compound.

Mature, field-collected boll weevils that had not been subjected to insecticides were used in these studies. They were fixed, embedded, and sectioned in a conventional manner, using Bouin's fixative followed by dehydration and clearing through a series of alcohols, acetone, cedarwood oil, and xylene, and finally embedding in Tissuemat. The microtome sections were cut with a rotary microtome to a thickness of 5 to 7 microns and were stained with a hematoxylin-eosin stain.

General Structure of the Alimentary Canal.—The small size of the boll weevil causes the alimentary canal to be thrown into various folds and loops. Nevertheless, it is divided into three regions—the foregut (stomodaeum), the midgut (mesenteron), and the hindgut (proctodaeum).

The foregut shows a greater specialization than appears in the immature forms of some insects, such as bollworm larvae. There are at least two distinct regions, the buccal cavity, pharynx, esophagus, and crop making up one, and the proventriculus the other.

The midgut appears as one uniform tube with little or no variation in size and cell structure.

In the hindgut there is little or no differentiation between the ileum and the colon. According to the terminology of Snodgrass (1935), this portion of the hindgut is the anterior intestine, and the rectal portion is the posterior intestine.

The Stomodaeum.—The stomodaeum of the boll weevil has two distinct regions, the fore part (fig. 1), consisting of the buccal cavity, pharnyx, esophagus, and crop, which are structurally the same except possibly in the length of the spines on the intima layer; and the hind part (fig. 2), which is the proventriculus and is a highly specialized part of the alimentary canal. Morphologically, however, the fore and hind parts are of the same origin.

The stomodaeal cell walls are of simple structure,

[1] Report of a study made under the Research and Marketing Act of 1946. Accepted for publication March 20, 1961.

[2] In cooperation with the Texas Agricultural Experiment Station. Author resigned March 1955.

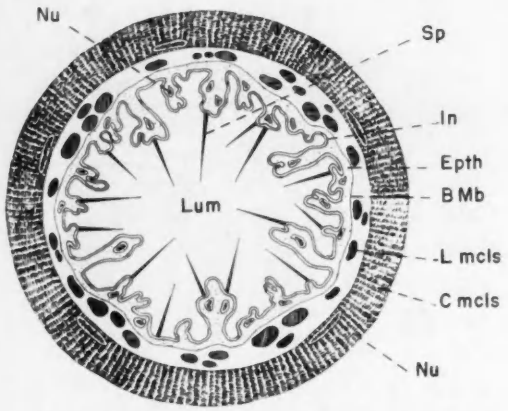

FIG. 1.—Cross section on anterior stomodaeum of the boll weevil.

and the epithelium is flat with indistinct cell boundaries. The basement membrane borders along the outer margins of these cells. The intima layer varies in thickness and is covered with long spines. On the proventriculus the spines are replaced with sclerotic plates. The epithelium and the intima layer are thrown into longitudinal folds, forward of the proventriculus, which follow no particular pattern. In the proventriculus there are eight longitudinal folds in a symmetrical arrangement.

In general the muscular arrangement of the foregut consists of an outer layer of circular muscle with an inner layer of longitudinal muscle fibers. They vary in size and thickness along different parts of the foregut. In the proventriculus there are two groups of longitudinal fibers between each fold.

The Mesenteron.—Two types of cells appear in the midgut of the boll weevil (fig. 3), columnar

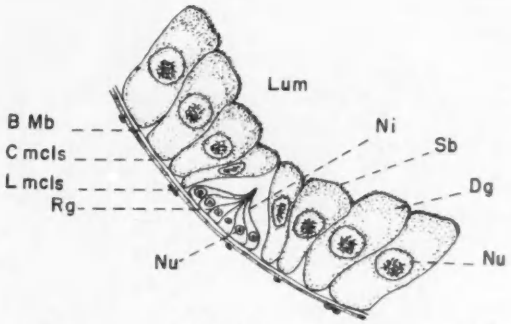

FIG. 3.—Cross section of the mesenteron of the boll weevil.

epithelial, or digestive cells, and regenerative cells. Most of the ventriculus is composed of digestive cells, which have a spongy appearance near their inner margins. They vary in shape and size, but the inner ends are exposed to the lumen of the gut. Along the inner margin of these cells lies the striated border. At irregular intervals between the digestive cells are small groups of regenerative cells, or nidi, the inner ends of which are not exposed to the lumen. The outer borders of both types of cells are limited by the basement membrane. No peritrophic membrane was present in the weevil gut.

In the ventriculus a thin layer of circular muscle is bordered externally by irregular groups of longitudinal muscular fibers.

The Proctodaeum.—The cell structure and the muscular arrangement of the anterior intestinal portion of the proctodaeum are similar to those of the anterior portion of the stomodaeum, except that the intima layer bears no spines (fig. 4).

The arrangement of the malpighian tubules (fig. 5) in respect to the posterior intestine supports Wigglesworth's hypothesis (1947) that some insects are capable of recovering water from the feces.

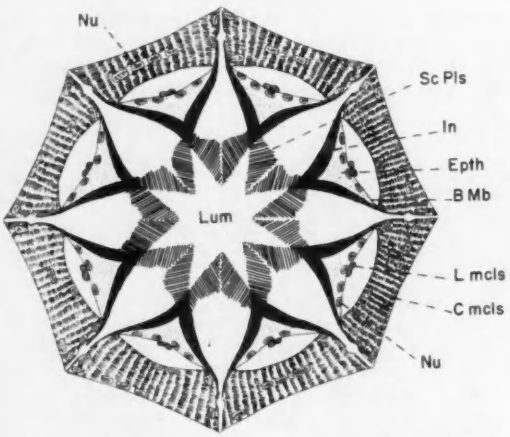

FIG. 2.—Cross section of the proventriculus of the boll weevil.

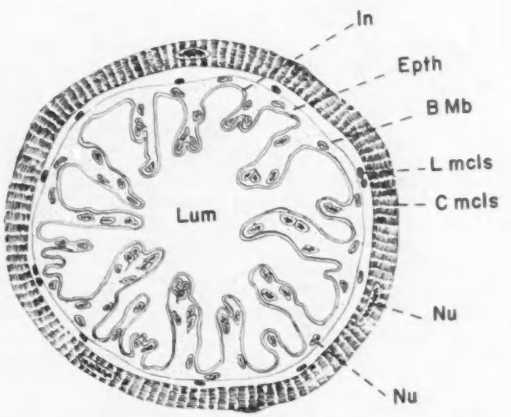

FIG. 4.—Cross section of the anterior intestinal portion of the proctodaeum of the boll weevil.

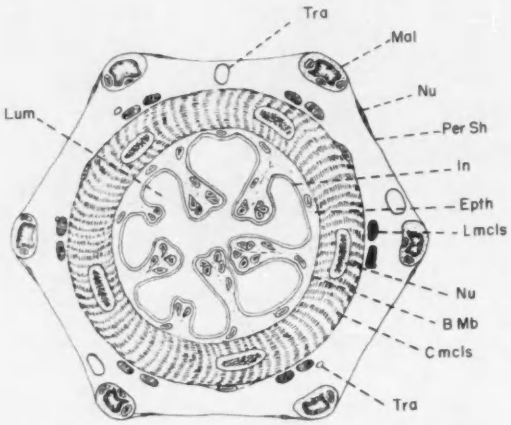

FIG. 5.—Cross section of the posterior intestinal portion of the proctodaeum of the boll weevil.

The epithelial cells still maintain their flat, indistinct, and primitive appearance; that is, there are no groups of specialized cells that may absorb water like those found in the honey bee. Moreover, the circular muscle is thick in the rectal area and this might also fail to support this theory. However, the fact that six malpighian tubules appear in symmetrical arrangement just outside this circular layer, and inside of what appears to be a double peritoneal sheath, adds weight to this hypothesis.

Briefly, the posterior intestine is made up of epithelial cells bordered internally by an intima layer and externally by a basement membrane, a strong circular muscle, six pairs of longitudinal muscle fibers, and six malpighian tubules in symmetrical arrangement; of tracheal branches; and of a double-layered peritoneal sheath with nuclei scattered between the layers.

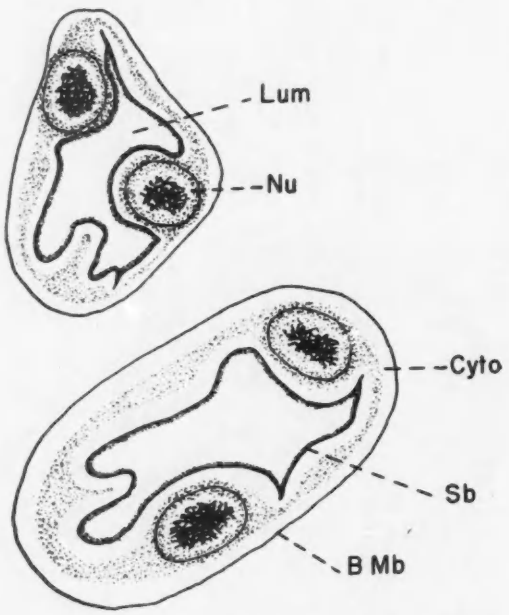

FIG. 6.—Cross section of Malpighian tubules of the boll weevil.

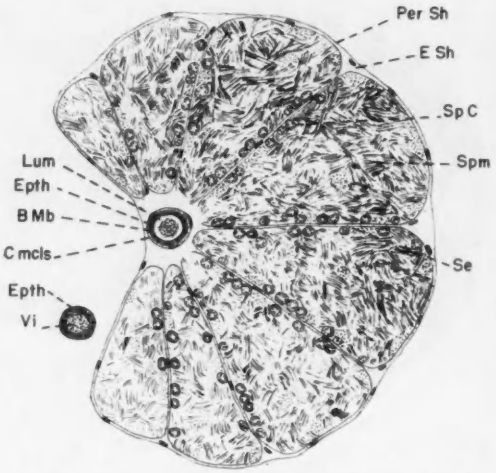

FIG. 7.—Cross section of testis and accessory gland of the boll weevil.

The Malpighian Tubules.—Malpighian tubules (fig. 6) are composed of only one type of epithelial cells having large nuclei and irregular inner margins with a fine striated border. Externally, the tubules have a basement membrane but no evidence of any muscle fibers; no differentiation of separate regions is noted.

The Male Reproductive Organs.—The testes (fig. 7) contain eight septa, all of which enter into a common duct, the vas deferens, no vas efferens being present. The septa are surrounded and separated from one another by an epithelial sheath, and the entire organ is invested in a peritoneal sheath. Sperm cysts are found along the walls of the septa, and sperm cells in various stages of development are found throughout each compartment.

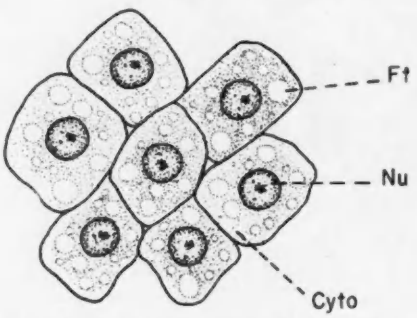

FIG. 8.—Cross section of the fat body of the boll weevil.

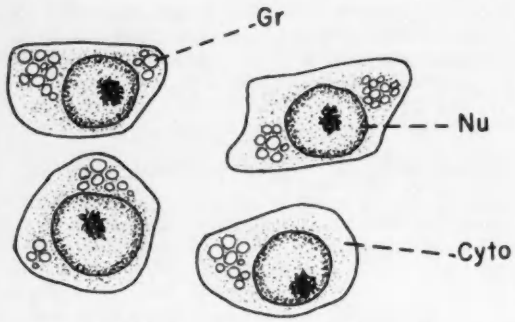

FIG. 9.—Oenocytes of the boll weevil.

The vas deferens leading from the testes is made up of a heavy, tightly packed layer of nucleated epithelial cells, surrounded by a basement membrane and strong circular muscle fibers. No epididymis is formed by the vas deferens in the weevil.

A pair of tubular accessory glands is associated with the testes. The tubes are made of closely nucleated epithelial cells, and the viscid substance in the center is very eosinophilic.

The Fat Body.—The fat body (fig. 8) is an aggregate of cells widely distributed throughout the organism. The cytoplasm is vacuolated where fat deposits were stored at the time of fixation. Nuclei are small and clearly visible.

The Oenocytes.—The oenocytes (fig. 9) are closely associated with the fat body, but they are much larger and stain more readily. The nuclei are large and stain very dark, whereas the cytoplasm is definitely eosinophilic. Fairly large granules appear in the cytoplasm of the oenocytes. There are considerably fewer oenocytes than fat cells, and they appear as separate cells rather than aggregate groups.

The Labial Glands.—The labial glands (fig. 10) are branched tubular glands situated on each side of the esophagus, progressing forward and slightly below the alimentary tract as straight tubes. They unite into a single median tube in the long proboscis of the weevil, opening near the tip. It is significant to note that labial glands have rarely been reported in adult Coleoptera.

Muscle and Attachment to Body Wall.—Two types of muscle insertions are described in figure 11. In one type the muscle is inserted into the coxa where definite connective fibrils, or tonofibrillae, are formed with the cuticle; in the other the muscle of the

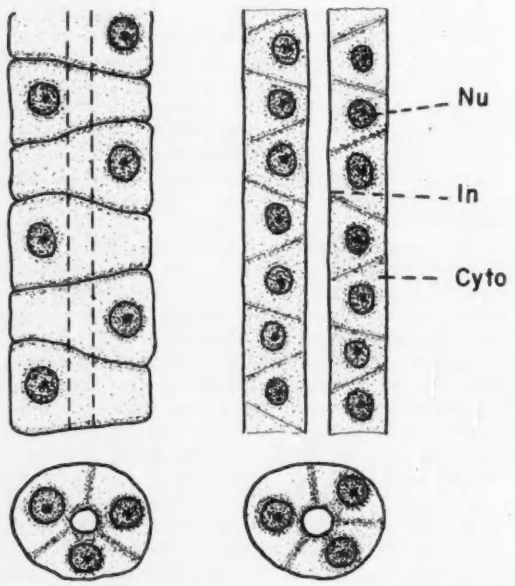

FIG. 10.—Longitudinal and cross section of labial glands of the boll weevil.

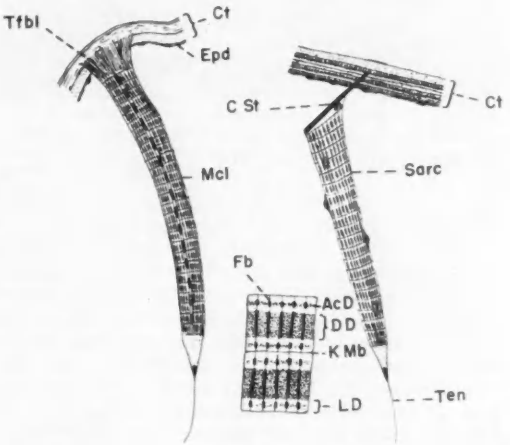

FIG. 11.—Longitudinal section of muscle of the boll weevil.

LIST OF ABBREVIATIONS

Ac D	Accessory disc	Lum	Lumen
B Mb	Basement membrane	Mal	Malpighian tubules
C mcls	Circular muscles	Mcl	Muscle
C St	Chitinous stalk	Ni	Nidi
Ct	Cuticle	Nu	Nucleus
Cyto	Cytoplasm	Per Sh	Peritoneal sheath
DD	Dark disc	Rg	Regenerative cells
Dg	Digestive cells	Sarc	Sarcoplasm
E Sh	Epithelial sheath	Sb	Striated border
Epd	Epidermis	Sc Pls	Sclerotized plates
Epth	Epithelium	Se	Septa
Fb	Fibrilla or sarcostyle	Sp	Spines
		Sp C	Sperm cyst
Ft	Fat deposit	Spm	Spermatozoa, developing and mature
Gr	Granules		
In	Intima		
K Mb	Krause's membrane	Ten	Tendon
		Tfbl	Tonofibrillae
LD	Light disc	Tra	Trachea
L mcls	Longitudinal muscles	Vi	Viscid substance

thorax is inserted on chitinous stalks, giving the appearance of apodemes.

The muscle fibers are made up of several fibrillae, or sarcostyles, and are contained in sarcoplasm. Two types of striated muscles are illustrated. In one type the nuclei pass through the center of the fibrils in a straight row, and in the other they are scattered on the outer surface in the sarcoplasm. Some of the muscles terminate in what appears to be an elongate cell containing a nucleus with a long tendon.

The telophragma, or Krause's membrane, is present as are also the dark and light discs, and an accessory disc. Henson's line and the median disc were not distinguishable.

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Nematode Parasites and Associates of the Smaller European Elm Bark Beetle, *Scolytus multistriatus* (Marsham)[1]

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ABSTRACT

In studies of nematodes associated with *Scolytus multistriatus* (Marsham) the following 12 genera were found: *Tylenchorhynchus, Ditylenchus, Deladenus, Bursaphelenchus, Aphelenchoides, Parasitaphelenchus, Chambersiella, Goodeyus, Cylindrocorpus, Poikilolaimus, Panagrobelus* and *Panagrolaimus*. Of these, only *Parasitaphelenchus oldhami* Rühm was parasitic; in this instance as larvae in the insect body cavity. The other genera were found in *S. multistriatus* egg galleries, in larval mines, and on the insect body. Every sample population of *S. multistriatus* examined was infested with *P. oldhami* whose incidence and magnitude appear density dependent. Life histories of both the parasite and its host are closely paralleled. Pathological effects of this nematode, if any, remain obscure.

Scolytus multistriatus (Marsham), the smaller European elm bark beetle, was reported as an important vector of the Dutch elm disease fungus in Europe (Fransen 1932) and in the United States (Collins et al. 1936). This beetle is probably the most important vector of the disease in the latter country. Discovery of the disease in Wisconsin (Anonymous 1956) prompted initiation of a research program to investigate various aspects of the problem.

One research approach has been a study of biotic agents affecting populations of the insect vectors. In early research at the University of Wisconsin, nematodes were found commonly in association with *S. multistriatus*, and studies were established to identify the nematodes and to investigate their interrelationships with the beetles.

METHODS AND MATERIALS

Beetle Rearings.—Beetles were reared using one female and two males as potential parents. These were released in 1-gallon ice cream cartons each containing an elm bolt approximately 6 inches long and 6 inches in diameter. The lid of the carton had a fabric top for aeration. Prior to beetle release, ends of the elm bolts were dipped in melted paraffin to prevent wood desiccation.

Brood and galleries from the rearings were examined during various stages of beetle development to correlate the life cycle of *Parasitaphelenchus oldhami* with that of its host, *S. multistriatus*. Some of the progeny were allowed to proceed to maturity to determine the effect of the nematode parasites on emergence of adult beetles.

Beetle Dissections.—Dissections were made in physiological saline solution to prevent distortion of the nemas. The quality of the dissecting solution may be enhanced by adding a small amount of wetting agent, such as Vatsol OS® (American Cyanamid Co.). Tap or distilled water diffuses rapidly into parasitic nematodes and may cause distortion, swelling or bursting of the specimens. Microscopic dissections of beetles were made under 20 to 120 magnifications.

Prior to dissection, the beetles were washed and examined for external nemas to prevent confusion of external with internal forms. In adult beetles, the elytra were removed for later examination. The specimen was opened dorsally and the gut removed for examination of the fat bodies, reproductive organs and body regions. Immediate examination of the body cavity is necessary if the nemas are to be found in their natural positions. Gut contents were examined later to avoid obscuring preceding observations.

In Vitro Nematode Rearings.—Colonies of several genera of nematodes found in beetle galleries were reared in vitro on a mixed culture of a fungus, *Graphium* sp., and a bacterium isolated from beetle galleries. Colonies were established by slicing small sections infested with nematodes from a newly

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. Portions of these data were presented at the joint meeting of the Entomological Societies of Ontario, Canada, and America held at Detroit, Michigan. November 30-December 3, 1959. Accepted for publication March 6, 1961.

formed beetle gallery and placing these on a medium of potato-dextrose-agar supplemented with ground elm twigs. The fungus and bacterium grew on this medium and furnished food for the nematodes.

Extraction of Nematodes from Bark Samples.—The apparatus used for extracting nemas from bark samples consists of a 3-inch rubber tube (bearing a pinchcock) attached to the spout of a 6-inch funnel.

The sample is washed in water with a stiff-bristle brush and the washings left standing for about 30 minutes to allow the nemas to settle. Nemas are concentrated into a smaller amount of water by repeated decanting until washings are containable in a 250 ml. beaker. Muslin is fastened over the beaker and it is inverted into a funnel partially filled with water. After 3 hours or longer a 10-ml. sample may be removed and examined for nemas.

If the rapid desiccation method of preparing nemas for permanent mounts (discussed later) is used, the sample may be taken from the funnel in a small preparation dish, thus eliminating individual handling of the nemas.

Rapid Desiccation Method.—The classical method of preserving nematodes in pure glycerol (Thorne, in press) yields suitable specimens, but requires a dehydration period of 4 to 6 weeks. A more rapid method has been developed which also yields specimens preserved in pure glycerol. The technique still involves ethyl alcohol as a carrier for glycerol. Nemas placed directly in glycerol collapse, as do those introduced directly into alcohol of concentrations above 20 percent. The modified procedure is as follows:

Nemas are killed and relaxed by heating gently in water or dissecting solution in a small preparation dish (37 mm. x 19 mm.) and fixed in Kahle's fixative (Peterson 1953). An aspirator with a finely drawn glass tip (figure 1-C) is useful for changing solutions. Kahle's fixative is aspirated and replaced with 2 to 3 ml. of a solution consisting of 20 parts of 95% ethyl alcohol, 1 part glycerol, and 74 parts distilled water. The small preparation dish containing nemas in the latter solution is placed in a larger preparation dish (63 mm. x 28 mm.) and the space between the dishes is filled with 95% ethyl alcohol (figure 1-A). After sealing the lid with petroleum jelly, the microchamber is placed in a temperature-controlled cabinet at 30° to 40° C. for 24 hours. (Optional: the nemas are now in a 95% ethyl alcohol solution, an ideal place for staining. The dyes must also be in 95% ethyl alcohol solution.) The concentrated solution is aspirated and replaced with about 5 ml. of a solution consisting of 95 parts of 95% ethyl alcohol and 5 parts glycerol. The small dish is partially closed with a lid (figure 1-B) and allowed to desiccate at room temperature or at 30° to 40° C. for at least 12 hours. The nemas can either be mounted immediately or placed in a desiccator.

Timing is not critical except in the desiccation step, which, if shortened, results in collapse of the nematodes. The entire process may be carried out at room temperature by increasing time intervals.

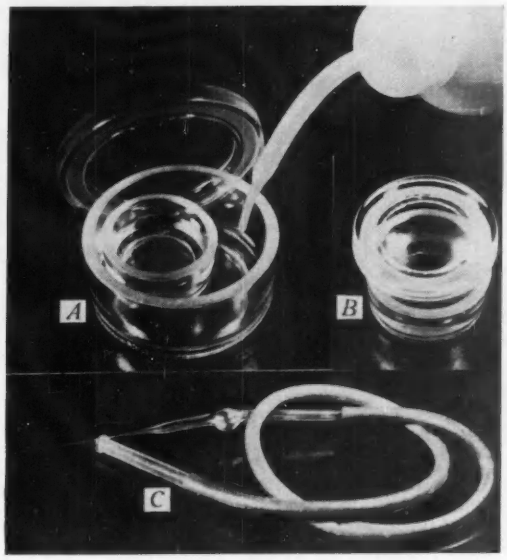

FIG. 1.

A, micro-chamber used in rapid desiccation method.
B, small preparation dish with lid slightly open to permit desiccation.
C, aspirator with finely drawn tip.

Preparation of Permanent Mounts.—All permanent mounts were made on Cobb metal slides (Cobb 1917). Nematodes mounted in this manner are suspended between two cover slips (one square, one round) and can be observed from either side. The procedure for making slide mounts is as follows.

Four to ten nemas of the same diameter are selected and lowered to the bottom of a drop of glycerol placed in the center of the square cover slip and three pieces of glass rod placed around them. A round cover slip is heated and lowered gently over the drop of glycerol. Excess glycerol which may occur around the edge of the cover slip should be blotted up (moist filter paper is satisfactory) and the slide ringed with "Zut" ringing compound (Thorne 1935).

DISCUSSION OF OBSERVED NEMATODES

To retain continuity of subject material, a discussion of the taxonomic categories involved in this study is combined with previous contributions to the study of associations between nematodes and insects.

Order TYLENCHIDA Thorne, 1949

Members may occur as free-living forms, or as plant or animal parasites. Parasitism in both plants and animals varies extensively. Free-living species are often found associated with bark beetles and other insects. They use the insects as a mode

of transportation, and derive their nutrition from either excrement or microorganisms growing in the insect habitat or possibly plant tissues.

Superfamily: Tylenchoidea Chitwood and Chitwood, 1937.

Certain members parasitize bark beetles and a variety of other Coleoptera. They are prevalent in tree-inhabiting insects and insects living on or in soil and decomposing organic matter.

Fertilized parasitic females usually attack the host and invade the body cavity, where the eggs are produced and larvae develop. Females may exhibit drastic parasitic adaptations with the body often becoming so modified that recognition as a nematode is difficult. The larvae leave the host through the anus or genital ducts, mature, mate and the female progeny re-infest the succeeding host generation.

Many of the Tylenchoidea, in feeding, may cause partial sterility of their insect host. This is probably brought about by withdrawal of food from the host tissues, which in turn arrests development of the gonads. The progeny are often reduced to one-third or one-half of the normal number.

Family **Tylenchidae** Filipjev, 1934

Genus *Tylenchorhynchus* Cobb, 1913.

Synonym: *Bitylenchus* Filipjev, 1934.

A few immature specimens were found in one tree. There is apparently only one previous mention of this genus associated with insects. Thorne (1935a) found *T. magnicauda* (Thorne) in frass of *Dendroctonus monticolae* Hopkins at the base of a tree. He believes it may have been a migrant from the soil, as he has since isolated the species from soil. The genus possesses a well developed stylet and is typically plant ectoparasitic.

Genus *Ditylenchus* Filipjev, 1934.

Ditylenchus are either plant parasitic or free-living. None is parasitic on insects, although about one-third of the described species inhabit tunnels of bark- and wood-boring beetles (Thorne, in press). One and possibly two species of the genus were abundant in the galleries of all *S. multistriatus* populations observed in Wisconsin. These were reared in vitro by previously discussed methods. Large masses of nemas congregated on the side and lid of the petri dish container within 10 to 14 days after inoculation with 25 to 50 adults. After the second moult, *Ditylenchus* pass into a resistant stage which is carried under the elytra.

Family **Neotylenchidae** Thorne, 1949

Genus *Deladenus* Thorne, 1941.

Species of this free-living genus have been reported from soil, decaying fungus, oak galls and beneath dead tree bark (Thorne 1941). The species in *S. multistriatus* galleries are probably transported by the beetles.

Superfamily: Aphelenchoidea Fuchs, 1937.

Species occur as body-cavity, gut, or external parasites, or as commensals. The infestive stage varies from parasitic females (*Sphaerularia*) to parasitic larvae (*Parasitaphelenchus*). Biology of species with parasitic adult females is similar to that of Tylenchoidea; eggs and young occur in the body cavity of the host. Temporary larval invasion usually causes negligible effect, indeed some populations infested with *Parasitaphelenchus* appear more vigorous than uninfested populations.

A specially adapted stage for being transported by insects occurs in some species. These are sometimes so drastically modified that identification by ordinary taxonomic characters is impossible. The nemas may have reduced mouth structures and may possess a water-repellent covering (Bovien 1937). These may be carried loosely on the insect integument or by a peduncle-like hold-fast extending from the mouth region. The hold-fast is a secretion and is not an integral part of the nema. Fuchs (1930) and Thorne (1935) reported some species under elytra to be protected by a cocoon-like structure.

Family **Aphelenchoidae** (Skarbilovich, 1947) Paramanov, 1953

Free-living forms, plant or insect parasites occur. Rühm (1956) erected a subfamily, Parasitaphelenchinae, encompassing four genera of insect associates which closely resemble members of the subfamily Aphelenchoidinae Skarbilovich, 1947. Goodey (1960) sank both subfamilies to redundancy.

Genus *Aphelenchoides* Fischer, 1894.

Synonyms: Subgenus *Chitinoaphelenchus* Micoletzky, 1922; *Pathoaphelenchus* (Cobb, 1927) Steiner, 1932.

This large and complex group, composed of obligate plant parasites, insect associates and free-living forms, has often been an unstable taxonomic catch-all. The genus has been a too-inclusive group and even now probably consists of several valid genera (Thorne, in press).

Three species of *Aphelenchoides* were under the elytra or in the galleries of *S. multistriatus*. There was no indication of parasitism. Apparently fungal mycelium is their food source as it was possible to rear them on *Graphium* sp. isolated from *S. multistriatus* galleries.

Genus *Bursaphelenchus* Fuchs, 1937.

There are about 20 species, all associated with insects, particularly Scolytidae. Males possess a flattened, sclerotized extension of the tail, which, although not a true bursa, is the basis for the generic name. Rühm (1956) reduced the genus to subgeneric status under *Aphelenchoides*, but most workers recognize it as a distinct genus.

Larval and adult forms found in *S. multistriatus* galleries and/or under the elytra were not parasites. As with *Aphelenchoides*, beetles furnish a mode of transportation.

Genus *Parasitaphelenchus* Fuchs, 1930.

Adults occur in beetle galleries but persist only a short time. Mature males and immature females are often found together because of earlier maturation of males, with greatest numbers of adults usually occurring about 2 weeks after emergence of beetle larva from the egg (Rühm, 1956). Larvae occur in the body cavity of many species of bark beetles and are difficult to differentiate if adult forms are not present.

Order RHABDITIDA Chitwood, 1933

Superfamily: Rhabditoidea (Orley, 1880) Travassos, 1930.

Rhabditoidea are nonspearbearing nemas occurring in soil, decomposing organic matter and as animal parasites. The only other superfamily, Drilonematoidea, is parasitic in the body cavity of earthworms.

Many Rhabditoidea are commensals of insects and express a phoretic relationship. The nemas may be attached loosely to the insect integument, more securely by an adhesive secretion, or carried under the elytra (Bovien 1937). The families Diplogasteridae Steiner, Cephalobidae Chitwood and Chitwood, Chambersiellidae (Thorne) Sanwall, Rhabditidae Orley, and Cylindrocorporidae Goodey all have species which are commensals with insects.

Körner (1954) found a species of Rhabditidae in the body cavity of a lucanid and Rühm (1956a) described a bark-beetle gut parasite. Théodoridés (1949) and Körner (1954) found Diplogasteridae attached internally to the genital segments of beetles. The Steinernematidae are all insect parasites, one of the more important genera being *Neoaplectana*. Carabonematidae was established by Stammer and Wachek (1952) and consists of the genus *Carabonema* Stammer and Wachek, found in the body cavity of carabid beetles.

Family **Chambersiellidae** (Thorne, 1937) Sanwal, 1957

Genus *Chambersiella* Cobb, 1920.

Cobb (1920) stated, "this genus comprises a considerable number of species," but he described only one. To the writers' knowledge, only one other species has been described. Sanwal (1957) described *C. bakeri* from bark of oak and established the monogeneric family Chambersiellidae.

The genus is free living; a striking feature being the large, powerful odontia used in feeding. The nemas probably are transported by insects but apparently have no effect on beetles sharing common galleries.

Family **Cylindrocorporidae** Goodey, 1939

Genus *Cylindrocorpus* Goodey, 1939.

Synonym: *Cylindrogaster* Goodey, 1927.

The species in these studies, *C. erectus*, was described from egg and larval galleries of *S. multistriatus* by Massey (1960). Apparently *C. erectus* feeds on fungi in the galleries. The nemas are probably carried by beetles from one tree to another. Other species are free living in a variety of habitats.

Genus *Goodeyus* Chitwood, 1933.

Fuchs (1933) found larvae under the elytra of *Scolytus scolytus* (Geoffroy) and Rühm (1956) found young on larvae, pupae and under the elytra of *Scolytus*. *Goodeyus* occurred sparingly beneath the bark of *Ulmus thomasii* (Sargent) attacked by *S. multistriatus* and *Hylurgopinus rufipes* (Eichhoff). These were mixed with *Cylindrocorpus*, a closely related genus.

Family **Rhabditidae** Orley, 1880

Genus *Poikilolaimus* Fuchs, 1930.

Rühm (1956) found individuals in frass of Cerambycidae in *Pinus silvestris* Linnaeus and *Picea excelsa* Link, and in frass of *Dryocoetes autographus* Ratzburg. He redescribed the genus and selected *P. piniperidae* Fuchs as a representative form. Specimens found in Wisconsin were taken from galleries of S. *multistriatus* in *Ulmus thomasii*.

Family **Cephalobidae,** Chitwood & McIntosh, 1934

Genus *Panagrolaimus* Fuchs, 1930.

Panagrolaimus is a cosmopolitan genus, inhabiting soil and decomposing organic material, and is a common inhabitant of bark beetle galleries. They occurred in *S. multistriatus* galleries at all locations examined in Wisconsin and were often the predominant nematode life present. They are transported under the elytra of associated beetles.

Genus *Panagrobelus* Thorne, 1939.

Little is known of this genus other than it occurs in bark beetle frass (Thorne, 1939). Three species have been described. Individuals are free living and have no apparent effect on associated beetles. The nemas are probably carried by adult beetles, although their presence on beetles was not established.

MORPHOLOGY, BIOLOGY, AND LIFE HISTORY OF *Parasitaphelenchus oldhami* RÜHM, 1956

Parasitaphelenchus oldhami was reported from the body cavity of *Scolytus scolytus* and *S. multistriatus* by J. N. Oldham (1930) in England. Oldham erred when he identified the larval parasite as an immature form of a *Parasitylenchus* adult female found in the body cavity of *S. scolytus*. He named the adult *Parasitylenchus scolyti*, which remains a valid name for that form. Rühm (1956) renamed the larval *Parasitaphelenchus*, *P. oldhami*, on the basis of larval characters.

Morphology.—Preadult parasitic larvae (figure 2). Length 0.62 to 0.80 mm. Width 0.030 to 0.041 mm. Cuticle thin, finely striate. Neck tapering gradually to a rounded lip region. Spear obscure or absent,

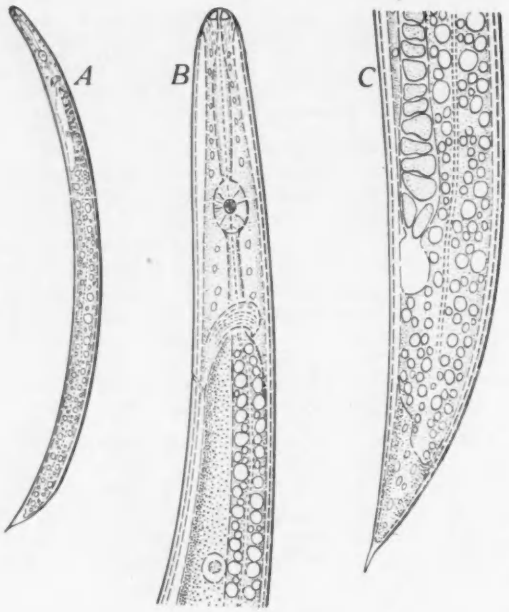

FIG. 2.
Parasitaphelenchus oldhami Rühm, 1956.
A, fourth stage (preadult) larva x 100.
B, anterior portion of body showing median bulb, nerve ring, excretory pore and anterior end of esophageal glands lying ventral to the intestine x 500.
C, posterior portion of body illustrating genital primordium x 500.

not observed. Esophagous narrow; 12 percent of body length. Median bulb ovoid; two-fifths as wide as neck and twice as long as wide. Nerve ring prominent; just above junction of esophagus with intestine. Excretory pore just before nerve ring. Salivary glands about four times as long as body width. Lumen of intestine appearing as a narrow tube. Intestine densely granular ending blindly. Anus and rectum not observed; probably nonexistent. Caudal portion of body somewhat hyaline. Genital primordium in posterior third of body. Posterior portion of body dorsally convex-conoid to a pointed, mucronate terminus. Mucro spicate; 8 to 10 microns long.

Life History.—The observed life history of *Parasitaphelenchus oldhami* closely paralleled that of its host (figure 3).

Parasites were present in a preadult (fourth-stage larval) stage when the beetles emerged. These preadult larvae exited from their host after the beetles attacked a new host tree. Nemas usually were absent or present in low numbers in adult beetles recaptured after mating and oviposition. Nemas left the host in a staggered manner, encompassing the time of beetle oviposition. These sexually immature nemas moult to adults in the beetle galleries.

Nema eggs hatched and the juveniles passed

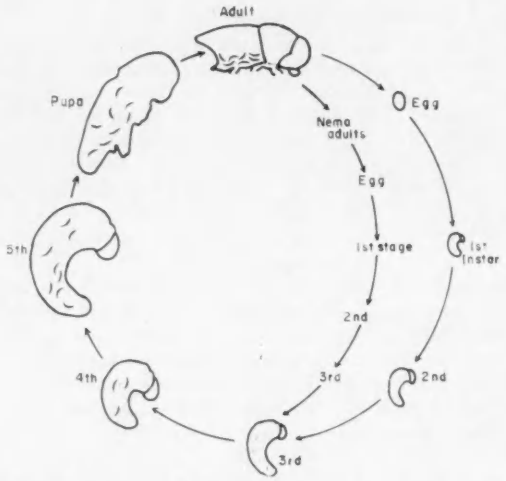

FIG. 3.—Comparative life cycles of *P. oldhami* and *S. multistriatus*.

rapidly through two stages before reaching the infestive third stage. It was not possible to ascertain whether the first two stages fed in the galleries as free-living forms. No feeding was apparent prior to parasitizing the beetle larvae. During the time required for the parasites to reach the infestive stage, the beetle larvae had usually progressed to the third or fourth instar. Nemas were not found parasitizing beetle eggs or first-instar larvae and occurred only once in second-instar larvae. Nemas became prevalent in the abdominal cavity of later-instar larvae.

Mode of entry into the host was not definitely determined; however, past workers (Fuchs 1938, Rühm 1956) indicated that entrance is probably by ingestion followed by penetration of the gut into the body cavity. If this is true, juveniles should be present in the alimentary tract of the beetle larvae. One thousand dissections of insect larval stages were made without finding parasites in the alimentary tract. For this reason, the validity of entrance by ingestion is questionable, but no more reasonable suggestion can be offered. The parasites may possibly penetrate the gut wall so rapidly after ingestion that this action escapes detection. Entrance by direct penetration through the integument, or indirectly through the trachea or the anus are considerations to explore.

The parasites remained in their host through the pupal stage and into the adult beetles. Moulting occurred once in the host body cavity, early during the parasitic phase before pupation of the host larvae. Rapid growth occurred during the first few days of their parasitic life, but little size or structural change was observed during the last larval, pupal or adult stages of the beetle. In beetle larvae and pupae, the nemas were scattered among the fat bodies throughout the body cavity; usually being surrounded by fat granules during the pupal stage. In the

adults the parasitic larvae migrated ventrally and posteriorly in the abdominal cavity.

Nemas passed through a quiescence during the overwintering period of the host larva. During the quiescence there were no morphological changes; however, the nemas often assumed a tightly coiled position. Activity resumed with renewal of feeding by its host in the spring. It appears that overwintering may also occur as dauerlarvae in galleries.

Biology.—The percentage of beetles parasitized varies with different ecological conditions. *Parasitaphelenchus* infestation of *S. multistriatus* populations in Wisconsin usually averaged from 75% to 100%, with a range from 10% to 100%. Male and female beetles were infested in about equal numbers. Oldham (1930) reported 60% infestation of both male and female beetles while Rühm (1956) reported only 10% infestation.

Nemas per infested beetle usually ranged from 30 to 40, but 100 to 200 per beetle were not uncommon in heavily infested beetle populations. Parasites can be easily overlooked in sparsely infested populations which have only one or two nemas per infested beetle.

Incidence and magnitude of nema infestation were shown to be density-dependent by crowding beetle populations in laboratory rearings. The infestation was increased from 10% up to 100% in two to three generations by placing an excess of beetles upon a limited supply of brood wood. Circumstantial evidence of this phenomenon was also noted in nature. In very heavy outbreaks of *S. multistriatus*, such as along the Root River in Racine County, Wisconsin, percentage of infestation of individual beetle broods ranged from 85% to 100%. Scattered locations where only one or two trees were attacked frequently had as low as 10% nema-infested beetles.

Infestation of the progeny from individual females of the same beetle population often varied; therefore, infestation of the brood is apparently dependent on infestation of the immediate parents. Migration of nemas between neighboring broods occurred only where excessive crowding and intermingling of beetle larvae occurred.

Definite pathological effects on beetle populations were not recognized either in nature or laboratory rearings. There was no apparent correlation between incidence of nema infestation and host prolificness. Rühm (1956) did not observe pathological effects resulting from *Parasitaphelenchus* parasitism; however, Oldham (1930) reported that about 40% of both *S. scolytus* and *S. multistriatus* were rendered sterile. He based his conclusions on reduction in size of the reproductive organs. This could not be detected in these studies. Some beetles had as many as 200 parasites in the abdominal cavity without apparent damage to the testes or ovaries. It is possible that reduction in size of the gonads observed by Oldham was due either to a more pathogenic parasite or to other factors.

Individual matings of adult beetles were made to determine if a reduction in the progeny of parasitized beetles existed. Beetles ranging from 100-percent infested to free were reared with no significant difference in the number of offspring.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Professor Gerald Thorne for his invaluable aid with the taxonomic studies and for criticizing the manuscript.

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A Revision of the Genus *Vacusus* Casey (Coleoptera: Anthicidae)[1]

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ABSTRACT

This New World genus is redefined, and all its known species are discussed or described and keyed. New species are: *V.* **aztecus** (Mexico), **villosus** (Mexico), **vulgaris** (Argentina and Bolivia), **antennatus** (northwestern Argentina), and **jamaicanus** (Jamaica). Eight species are transferred from *Anthicus* to *Vacusus*, namely, *infernus* Laf. (=?*dugesi* Pic), *apicicornis* Laf. (=*plaumanni* Heberdey), *chilensis* Solier, *fulvonotatus* Laf., *peruvianus* Pic., *parvus* Pic (=*parvus* var. *atratulus* Pic and *weiseri* Pic), *subbrunneipennis* Pic, and *holoxanthus* Fairm. & Germ. Other new synonymy: *V. vicinus* (Laf.) (=*V. supplex* Casey and *V. monitor* Casey); *V. formicetorum* (Wasm.) (=*V. desertorum* Casey, =*V. prominens* Casey, =*V. suspectus* Casey); *V. nigritulus* (LeConte) (=*V. arcanus* Casey).

Casey proposed the generic name *Vacusus* in 1895 to include 10 Nearctic species, 6 of which he described as new. Pic (1911) soon relegated it to the rank of subgenus, and it is so listed in the Leng catalog. No additional species have been referred to it since 1895.

Actually, a number of Neotropical species are probably related to the species Casey brought together under this name. The present author prefers to treat all together, and to re-elevate the name *Vacusus* to the generic level. Pic apparently objected to Casey's generic names in most cases because they could be applied only to the members of the Nearctic fauna. Once a broader basis for delimiting a group has been realized, this objection loses its validity.

Vacusus is here defined as including all New World Anthicidae with the following characteristics: 1) Mesosternum expanded laterally into a broad plate, with rounded edges. 2) Right mandible with a single to triple tooth in the middle of the cutting edge, not a rasp as in *Acanthinus*. 3) Sides of prothorax almost straight, not deeply constricted near base as in *Formicilla* and many species of *Acanthinus*. 4) Surface generally shiny, with fine punctures, weak sculpture, sparse to very sparse pubescence, and usually with long, erect, tactile setae. 5) Last visible abdominal sternum of male (hypopygium) bilobed, the lobes downcurved and deeply grooved medially as in *Formicilla* and *Acanthinus*. 6) Aedeagus with a simple tegmen, a characteristic shared with many other genera.

The author has seen no Old World species that could be assigned to the genus as it is at present defined. The main problem, which cannot be resolved until a much larger portion of the World anthicid fauna has been studied in detail, is that the male genitalia are so different in the different sections of the genus that some doubt must exist as to the close relationship of the various species. *Vacusus vicinus*, which is the type species, *V. confinis*, and *V. infernus* differ from all the others in having a slightly asymmetrical sclerotized piece at the primary gonopore. Should division of the genus become necessary, these three species form a closely knit group and would remain together.

The key to species has been divided on a geographic basis for greater simplicity. One that included both North and South American species would, of necessity, include greater reference to the characters of the male genitalia. Samples from northern South America should probably be tried out in both divisions. In no case should Neotropical samples be more than tentatively identified until the genitalic characters have been checked.

KEY TO THE SPECIES OF *Vacusus* CASEY

North and Central America and West Indies

1. Setae of marginal fringe of mesosternum decumbent but not appressed to mesepisterna, and surpassing their lateral edges 2
   Setae of marginal fringe of mesosternum appressed to mesepisterna and not surpassing their lateral edges 4

[1] Accepted for publication March 6, 1961.

[2] The 1948 field work in Mexico and the southwestern United States was made possible by a grant-in-aid from the Society of the Sigma Xi. The Nearctic species were originally treated in a Ph.D. thesis submitted to Harvard University in 1950. The curators of most of the insect collections of the United States, and of the British Museum (Natural History), Museum G. Frey, Instituto Miguel Lillo, and Museo de La Plata, have been most generous in their loan of specimens. Their help is gratefully acknowledged.

2(1). Surface of head finely strigulose between punctures. Color uniformly piceous to almost luteous. British Columbia to central California ..... ***nigritulus*** (Lec.)
Surface of head smooth and shiny between punctures 3

3(2). Pubescence, other than tactile setae, decumbent and straight. Color usually luteous, varying to ferrugineous. Western U. S. and S. Canada ..... ***formicetorum*** (Wasm.)
Pubescence only subdecumbent, curved, rather long and shaggy. Color ferrugineous. Southeastern Mexico ..... ***villosus*** sp. n.

4(1). Upper side unevenly colored, the elytra with contrasting pale and dark markings. (Specimens from southeastern U. S. may be almost all dark, but usually show indications of paler elytral markings.) S. E. U. S. to E. Texas, and S. W. U. S. to Panama, and Greater Antilles ..... ***vicinus*** (Laf.)
Upper side uniformly colored, either pale or dark. (No species known from southeastern U. S. to E. Texas) 5

5(4). General color luteous. Slender, small, with truncate head and appressed pubescence. Jamaica ..... ***jamaicanus*** sp. n.
General color black, piceous, or (rarely) rufous 6

6(5). Surface of body, elytra, and legs finely but distinctly, and usually deeply, microreticulate. Color usually an intense black, often with a bronze or green cast. Elytral pubescence very short and appressed. S. and W. Mexico ..... ***infernus*** (Laf.)
Upper surface smooth between punctures 7

7(6). Head subtruncate at base; prothorax not flared anteriorly. Black to piceous, often with underside and legs slightly paler. Pubescence short and inconspicuous, not conspicuously paler than the ground color. Very abundant in southwestern U. S., ranging east to W. Texas and south to S. Sonora and Baja California Sur ..... ***confinis*** (Lec.)
Head almost evenly rounded behind eyes; prothorax flared anterolaterally; pubescence contrastingly paler than ground color. S. portion of Central Plateau of Mexico ..... ***aztecus*** sp. n.

South America

1. Setae or marginal fringe of mesosternum decumbent and surpassing lateral edges of mesepisterna, or mesosternum not fringed and mesepisterna with long fringes 2
Setae of marginal fringe of mesosternum appressed to mesepisterna and not surpassing their lateral edges 3

2(1). Mesosternum without a fringe, but mesepisterna with long fringes of setae, partly visible from above. Surface of head finely strigulose. Rather densely punctured and pubescent for members of this genus; elytra with a dark color pattern. Last antennal segment pale. Trinidad and Colombia to N. Argentina ..... ***apicicornis*** (Laf.)
Mesosternum with a long fringe. Shiny and smooth; head usually dark, prothorax pale, and elytra usually dark, but sometimes pale at base or all pale. Pubescence short and sparse. N. Argentina to Mato Grosso, Brazil ..... ***parvus*** (Pic)

3(1). Upper side uniformly colored, the elytra not marked with darker or lighter, or at most with base of elytra obscurely paler 4
Elytra with dark markings that partly or completely enclose a subapical pale spot on each 8

4(3). Black to rufous 5
Luteous. Small, slender species 7

5(4). Upper surface finely but distinctly microreticulate. Uniformly black. One specimen from Peru ..... ***infernus*** (Laf.) ?
Upper surface smooth between punctures 6

6(5). Entirely black and shiny. A single female specimen from Peru, probably of an undescribed species ..... sp. ?
Rufous to brunnescent, not notably shiny; elytra sometimes paler across base. N. Argentina and Bolivia ..... ***subbrunneipennis*** (Pic)

7(4). Tactile setae very short, hardly discernible, ca. 0.03 mm. long. Central Chile and W. Argentina ..... ***holoxanthus*** (Fairm. and Germ.)
Tactile setae obvious, ca. 0.06 mm. long. S. Brazil. Several female specimens of an undescribed species ..... sp.

8(3). Punctures on front of head moderately deep. Head and pronotum rufous. Elytral pubescence short and appressed, tactile setae long and erect. Peru ..... ***peruvianus*** (Pic)
Punctures on front of head very fine and indistinct. Head darker than pronotum in most specimens 9

9(8). Elytral pubescence decumbent, inconspicuous, touching or nearly touching surface at apex over most of elytra 10
Elytral pubescence subdecumbent, well away from surface at apex 11

10(9). Head and prothorax not unusually slender, the head often subtruncate at base. N. Argentina to Bolivia ..... ***vulagris*** sp. n.
Head and prothorax unusually slender, the head evenly rounded behind eyes. Known only from the unique female type. Colombia. ..... ***fulvonotatus*** (Laf.)

11(9). Elytral pubescence long and shaggy. Antennae not notably slender. Chile. ..... ***chilensis*** (Solier)
Elytral pubescence short. Antennae notably long and slender. Northwestern Argentina. ..... ***antennatus*** sp. n.

Genus ***Vacusus*** Casey

Vacusus Casey, 1895, Ann. New York Acad. Sci. 8: 674. *Anthicus* (*Vacusus*), Pic, 1911, Coleopterorum Catalogus, pars 36: 30. Leng, 1920, Catalog of the Coleoptera of America North of Mexico: 163. Blackwelder, 1945, U. S. Natl. Mus. Bull. 185(3): 433.

Type of genus: *Anthicus laetus* LaFerté, 1848, Monogr. *Anthicus*: 157, = *vicinus* LaFerté, op. cit.: 157, by present designation.

Vacusus vicinus (LaFerté)

(Fig. 1)

Anthicus vicinus LaFerté, 1848, Monogr. Anthicus: 157–8. LeConte, 1852, Proc. Acad. Nat. Sci. Philadelphia 6: 98. Champion, 1890, Biol. Cent.-Am., Coleop. 4(2): 242, pl. 10, fig. 23. Wolcott, 1936, Jour. Agric. Univ. Puerto Rico 20: 209. (Type locality: États-Unis d'Amérique - Dejean Coll. ex M. Lecomte. If the Lecomte referred to was John Eatton LeConte, the type locality is probably Liberty County, Georgia. However, Wolcott, loc. cit., states that it is actually Puerto Rico. He gives no supporting evidence.) Pic, 1911, Coleop. Cat., pars 36: 79, in part; Blackwelder, 1945, U. S. Natl. Mus. Bull. 185(3): 435, in part; in both these cases references to specimens from Argentina are in error, specimens identi-

fied as *vicinus* from that country really belonging to *vulgaris*, described in the present paper.

Anthicus loetus LaFerté, 1848, op. cit.: 157. (Type locality: Texas, Reiche Coll.). Spelling emended to *laetus* on same page, in *vicinus* discussion; on p. 315, in the Tabula Analytica; and on p. 329, Table Alphabétique.

Anthicus thoracicus LaFerté, 1848, op. cit.: 158–9. (Type locality: Nouvelle Orléans.)

Anthicus fulvomicans Quedenfeldt, 1886, Berliner Ent. Zeitschr. 30: 122–3. (Type locality: Puerto Rico.)

Vacusus laetus, Casey, 1895, Ann. New York Acad. Sci. 8: 676.

Vacusus supplex Casey, 1895, op. cit.: 676–7. (Type locality: Tucson, Arizona.) NEW SYNONYMY.

Vacusus monitor Casey, 1895, op. cit.: 677–8. (Type locality: Morgan City, Louisiana, or Galveston, Texas.) NEW SYNONYMY.

This species is variable in color. In Mexico, Central America and the West Indies most specimens are from luteous to rufous, the elytra being marked with a submedian, interrupted, dark band and dark apex. The dark markings on the elytra are reduced in most specimens from northwestern Mexico and southwestern United States. Specimens from southeastern United States are usually much darker than those from other areas and may be mostly piceous. But even the darkest specimens usually show some evidence of pale markings on the elytra when light is passed through these structures.

A male specimen from Lucedale, Mississippi is 2.77 mm. long, 0.50 wide at head, 0.52 prothorax and 0.83 elytra. Head subquadrate, 0.50 mm. long to base of clypeus; front flattened; surface shiny, smooth except for some fine transverse rugulae in region of eyes and behind clypeus; punctures moderately sparse and small, except for those bearing tactile setae, which are larger and shallow; pubescence sparse, short, appressed, fine, directed medially; tactile setae moderately long, fine, erect. Eyes moderately prominent, 0.22 x 0.16 mm. Frontoclypeal suture fine but distinct; clypeus wide, with surface like head, a few rugulae at base; labrum quite small, punctulate. Last segment of maxillary palpi obliquely truncate, not very large. Antennae moderately slender, gradually thickened apically; measurements (in 0.01 mm., base to apex, length over maximum width): 15/7, 8/5, 9/5, 9/6, 9/6, 9/7, 9/8, 9/8, 9/9, 9/9, 14/9. Segment I subparallel, pedunculate; II-V thickened apically, truncate at base; VIII-X quadrate, strongly truncate at base; XI truncate at base, tapered to a blunt point from about the middle.

Prothorax widest at 0.7 from base, the sides rounded anteriorly, almost straight behind, to a feeble basal constriction, 0.66 mm. long; width, collar 0.21 mm., widest 0.52, base 0.40. Collar and basal impressed line distinct, the latter with a row of small punctures in the bottom. Disc semiflattened, shiny, with some very feeble, confused strigulation anteriorly; punctures similar to those on head; pubescence and tactile setae also similar to those on head, the former directed backward. Elytra parallel, flattened, 1.61 mm. long, 0.66 wide across humeri and 0.83 at widest; humeri distinct; omoplates feebly and broadly elevated. Surface moderately densely, evenly punctured, the pubescence a little longer and less appressed than on prothorax. Pubescence ca. 0.04 mm. long, arising from punctures ca. 0.05 mm. apart; tactile setae ca. 0.06 mm. long, almost erect. Underside microreticulate, the thorax strongly, the abdomen feebly. Mesosternum, except on lateral edges, metepisterna except along ventral margin, and metasternum moderately densely, deeply punctured; punctures denser near sides of mesosternum, on metepisterna, and on anterolateral portions of metasternum; sides of prothorax longitudinally strigulate. Abdomen densely, finely punctured, with fairly dense appressed pubescence. Front femora stout, 0.55 x 0.22 mm.; front tibiae stout, 0.47 mm. long, straight, with short, dense pubescence beyond the middle of the flexor surface. Front tarsal segments I-IV broadened, with a dense ventral pad. Hind femora slender at base, thickened beyond middle, 0.67 x 0.18 mm. Pygidium 0.27 mm. wide, produced into a broad point, flat, deeply microreticulate with some small punctures and short setae near the beaded edge. Hypopygium bilobed, the lobes downcurved and channeled medially; it can be retracted into the sternum before it.

In the female the front femora are not so stout, ca. 0.33 as wide as long, in contrast to ca. 0.38 in the male, and the front tarsi are not expanded. The size varies from 2.35 to 2.75 mm. in the specimens measured. Variation in color has been discussed above. There seems to be little variation in shape and proportion, although darker specimens appear to be narrower when they are examined casually.

The aedeagus has the tegmen evenly tapered to a slightly downcurved point, which is higher than wide. The internal sac has some indefinite spines on it, mixed with the wrinkles and difficult to delimit. The gonopore armature consists of a single sclerotized piece of somewhat irregular shape, quite unlike any seen in other genera. How the ductus ejaculatorius associates with this sclerotized piece has not been determined.

The known range extends from southeastern and southwestern United States to Panama and includes the islands of the Greater Antilles in the West Indies. Specimens have been examined from the following localities.

UNITED STATES.—*North Carolina:* Raleigh. *South Carolina:* Clemson. *Georgia:* St. Simons Island. *Florida:* Tampa; Enterprise. *Alabama:* Mobile. *Louisiana:* Tallulah; Gueydon; Bayou Sara; Welsh; Harahan, Jefferson Par. *Arkansas:* Hope. *Mississippi:* Agricultural College. *Texas:* Beaumont; Lolita; LaBelle; San Antonio; Victoria; Laredo; Lee Co.; Brownsville. *Arizona:* Tucson; Phoenix; Globe; Yavapai Co. *California:* Blythe.

MEXICO.—*Baja California:* 6 mi. S.W. of Santiago. *Sinaloa:* Mazatlán; Bamoa. *Coahuila:* 10 mi. S. Allende. *Nuevo León:* Monterrey; Barretal. *Tamaulipas:* 14 km. S. Villagran (Rt. 1, km. 801). *San Luis Potosí:* 54 km. S. Tamazunchale, 4860′ (Rt. 1,

km. 311). *Michoacán:* Mil Cumbres, 7600′; Chavinda, 5110′. *Mexico:* Texcoco, 7200′. *Morelos:* 7 km. S. Cuernavaca. *Veracruz:* El Palmar, 17 km. W. Tetzonapa; San Rafael Jicaltepec.

CENTRAL AMERICA.—*Guatemala:* Agua Caliente; Los Amates; El Rancho (Kellerman). *British Honduras:* M-tee District (Peck). *Honduras:* La Ceiba; 12 km. W. Olanchito (E. C. Becker); Lancetilla (Stadelman). *Costa Rica:* San José (Nevermann); 8 km. W. José. *Canal Zone:* Barro Colorado; Ancon; Tabernilla; La Chorrera; Corozal.

WEST INDIES.—*Cuba:* Soledad, Cienfuegos; Havana; Jatibonica; Caymas; Baraguá. *Jamaica:* Spanish Town; Morant Bay; Clarkstown; Milk River; Santa Cruz; Trinityville; Bath St. Thomas (all E. A. Chapin and R. E. Blackwelder, Feb. 1937). *Hispaniola:* San Christobal, D. R. *Puerto Rico:* La Guanica; Salinas; Sabena Grande; Ensenada; Lajas; Mayaguéz.

Vacusus confinis (LeConte)

Anthicus confinis LeConte, 1851, Ann. Lyc. Nat. Hist. New York 5: 153; 1852, Proc. Acad. Nat. Sci. Philadelphia 6: 98. Champion, 1890, Biol. Cent.-Am., Coleop. 4(2): 243–4. (Type locality: seashore of San Diego, California.)

Vacusus confinis, Casey, 1895, Ann. New York Acad. Sci. 8: 678.

Upper surface entirely piceous to black, the under surface and legs usually slightly paler. Structurally almost identical with *Vacusus vicinus.* Measurements are not given for *confinis*; they seem to be extremely close to those for *vicinus* and are probably not different in any significant way. The aedeagus also is so similar that drawings of the two could not be distinguished with certainty.

This is one of the most abundant members of the family in the Sonoran Desert region in southern California and Arizona; it extends into the Rio Grande Valley in Texas but seems to be much less abundant there. Southward it is abundant at least as far south as Guaymas, Sonora, where it occurs with the closely related *V. infernus.* It is much less abundant in southern Sonora. Northward, populations decrease rapidly at about mid-elevations in Arizona and the northernmost records in California are from Tulare County. A single specimen from northern Idaho indicates that the species is able to survive well out of the range where it is abundant, perhaps on a temporary basis.

Specimens of *vicinus* from southeastern United States, including eastern Texas, are sometimes almost as dark as normal *confinis* specimens. These specimens practically always show some sign of pale markings on the elytra, at least when light is passed through them. These southeastern populations could be thought of as intermediate between lightly colored Mexican populations of *vicinus* and populations of *confinis.* However, this possibility is contraindicated by the presence of lightly marked populations of *vicinus* in the Sonoran Desert, with no indication of intergradation with the dark *confinis* populations. The *vicinus* populations are much less numerous in individuals than are those of *confinis* in the Sonoran Desert.

In the following list of localities, populations from along the south and southeast fringe of the range that are represented by only one or a few specimens have not been included. A short series of *vicinus* from Laredo, Webb Co., Texas, has most individuals dark, but one with an indication of pale banding on the elytra. Specimens have been examined from the following localities.

UNITED STATES.—*California:* Kaweah, Tulare Co.; Palm Springs; San Marino; Victorville; El Centro; Mt. Wilson, 6000′; Watsonville; Poway; Pasadena; and Coachella. *Arizona:* Yuma and Ehrenburg east to Willcox and San Bernardino Ranch, north to Showlow, very abundant in lowland areas, scarce to absent in southeastern mountains above about 5000′ elevation. *New Mexico:* Lordsburg; Deming; Carlsbad Caverns. *Texas:* McNary, Hudspeth Co.; Davis Mountains, Jeff Davis Co.; Porvenir, Presidio Co.; Tornilla Flat, Big Bend National Park; Alpine, Brewster Co.; and Sanderson, Terrell Co. *Idaho:* 1 spm. Slate Cr. R. S. (W. F. Barr).

MEXICO.—*Sonora:* 5 mi. N. Guaymas; 10 mi. E. Navajoa. *Baja California:* Purísima (W. M. Mann).

Vacusus infernus (LaFerté)

Anthicus infernus LaFerté, 1848, Monogr. *Anthicus:* 159. Champion, 1890, Biol. Cent.-Am., Coleop. 4(2): 243. (Type locality: Mexico.)

? *Anthicus dugesi* Pic, 1901, Ann. Soc. Ent. Belgique 45: 90. (Type locality: Tupataro, Mexico.)

While this species is very distinct from *vicinus* and *confinis,* it is closely related. From either it can be distinguished by its uniformly dark color, usually intense black, with bronze or greenish reflections, on legs and underside as well as on dorsal surface. The upperside is deeply and very distinctly microreticulate, as is the underside. In *vicinus* and *confinis* the underside is microreticulate, but not so deeply as in this species. The average size is somewhat larger than in the other two species mentioned, ranging from 2.70 to 3.21 mm. in the specimens examined.

A male from Texroco, Mexico, is 2.90 mm. long; head 0.52, prothorax 0.69 and elytra 1.69. The head is 0.54 mm. wide across eyes, 0.51 behind; prothorax 0.22 wide at collar, 0.55 maximum and 0.44 at base; elytra 0.64 wide at humeri, 0.84 maximum. Eyes 0.19 x 0.13 mm. Antennal measurements: 13/8, 10/5, 10/5, 10/6, 10/6, 10/7, 10/8, 10/8, 10/8, 10/8, 18/8. Front femora 0.54 x 0.20 mm.; protibiae 0.41 mm. and hind femora 0.64 x 0.18. Pygidium 0.33 mm. wide. Elytral pubescence ca. 0.03 mm. long; tactile setae ca. 0.05; punctures separated by ca. 0.04 mm. Aedeagus almost identical with that of *vicinus.*

The known range falls entirely within Mexico, except for a single female specimen from Peru which is doubtfully assigned here because of the extent to which it is isolated from the rest of the known range.

MEXICO.—*Sonora:* 5 mi. N. Guaymas; Navajoa;

10 mi. E. Navajoa; Alamos. *Sinaloa:* Los Mochis. *Jalisco:* 20 km. W. Lagos, 6130′. *Mexico:* Texcoco, 7200′.

PERU—? Tingo Maria, Monson Valley, IX-23-1954 (E. I. Schlinger and E. S. Ross), in California Academy of Sciences.

The Sonora specimens were taken from light traps, and the series from Texcoco on the ground during the daytime.

Vacusus formicetorum (Wasmann)

(Fig. 2)

Anthicus formicetorum Wasmann, 1894, Krit. Verz. Myrm. Term. Arthr.: 221. (Type locality: Garland, Colorado.)

Vacusus formicetorum, Casey, 1895, Ann. New York Acad. Sci. 8: 681–2.

Vacusus desertorum Casey, 1895, op. cit.: 680. (Type locality: Holbrook, Arizona.) NEW SYNONYMY.

Vacusus prominens Casey, 1895, op. cit.: 680-1. (Type locality: Peach Springs, Arizona.) NEW SYNONYMY.

Vacusus suspectus Casey, 1895, op. cit.: 682. (Type locality: Colorado?.) NEW SYNONYMY.

Ferrugineous, varying to luteous; front portion of pronotum somewhat flared at sides; head without fine wrinkles; length 2.0 to 2.5 mm. This species is not abundant, but has been taken in widely separated localities in western United States.

A male from Garland, Colorado, and probably from the type collection, is 2.14 mm. long. Head 0.40 mm. long, 0.45 wide across eyes, 0.44 behind, subquadrate, with sides almost parallel behind eyes, temporal angles rounded, base almost transverse; disc not flattened; surface smooth, shiny, with moderately dense, fine but deep punctures, bearing decumbent, moderately long pubescence, which is directed medioposteriorly; there is a small triangular impuncate area just behind the clypeus. Eyes 0.15 x 0.10 mm., oblique, subtriangular. Frontoclypeal suture indistinct. Antennae thickened from segment VII. Segment I stout, parallelsided, pedunculate; II-VI thickest near apex; VII-X subtriangular, truncate at base, X almost quadrate; XI stout, truncate at base, tapering to a blunt point from about the middle. Measurements: 12/6, 7/5, 7/5, 6/5, 7/5, 7/5, 7/6, 7/6, 7/8, 7/9, 12/7.

Prothorax 0.50 mm. long, 0.15 wide at collar, 0.47 maximum and 0.36 at base, broadest near front, the lateral expansions feebly tuberculate posteriorly, the sides feebly concave. Disc semiflattened, about as densely punctured as head but punctures broader, craterlike; pubescence decumbent, directed backward; tactile setae erect, about as long as pubescence. Collar very short; basal impressed line distinct. Elytra parallel, 1.24 mm. long, 0.49 wide at humeri and 0.67 at widest, the disc flattened. Humeri distinct; transverse impression not evident. Surface slightly roughened, with quite dense, small punctures, ca. 0.03 mm. apart. Pubescence decumbent, ca. 0.08 mm. long; tactile setae erect, also ca. 0.08 mm. Mesosternum, except for a narrow smooth edge on the expansions, with large, craterlike punctures and rugulae; metepisterna also deeply punctured except along ventral edges. Metasternum smooth, with moderately dense, small punctures. Abdomen very finely punctured, densely on first visible sternum, very densely on rest, with fine, appressed pubescence. Front femora quite stout, 0.38 x 0.15 mm.; front tibiae 0.33 mm.; hind femora 0.46 x 0.12. Pygidium 0.26 mm. wide, ogival, the apex slightly produced and the edges beaded; flattened, transversely strigulate, with a few small punctures and setae near apex.

The female has the front femora slightly more slender than the male. Measured specimens range from 2.0 to 2.5 mm. long.

The aedeagus is dorsoventrally flattened, with the sides of the tegmen tapering to a subapical constriction that sets off a small apical expansion. The internal sac bears a few small spines and the gonopore armature is simple, apparently consisting only of a slight sclerotization of the tip of the ductus ejaculatorius.

Specimens have been examined from the following localities.

UNITED STATES.—*Arizona:* Galiuro Mtn.; Texas Pass, Dragoon Mts.; Wickenburg; Globe; Pinal Mts.; Holbrook; and Peach Spring. *Colorado:* Garland; Cañon City, 5800′. *Nevada:* Esmeralda Co. *South Dakota:* Fox Ridge.

CANADA.—*Alberta:* Medicine Hat (J. Carr).

The type series was collected by E. A. Schwarz in the nest of an ant identified as *Formica obscuripes.* Part of the original series is in the Museum of Comparative Zoology, Harvard University. Subsequently collected specimens have not been taken with ants, as far as the labels indicate; a few have been taken at light or in light traps.

Vacusus nigritulus (LeConte)

(Fig. 3)

Anthicus nigritulus LeConte, 1851, Ann. Lyc. Nat. Hist. New York 5: 154; 1852, Proc. Acad. Nat. Sci. Philadelphia 6: 101. (Type locality: San Francisco, California.)

Vacusus nigritulus, Casey, 1895, Ann. New York Acad. Sci. 8: 678–9.

Vacusus arcanus Casey, 1895, op. cit.: 679–80. (Type locality: Yakima, Washington.) NEW SYNONYMY.

An entirely piceous, shiny species, easily distinguished from other Nearctic species of *Vacusus* by its moderately abundant, subdecumbent pubescence and the presence of longitudinal strigules on the head. In general aspect it resembles *Anthicus obscurellus* LeConte, of the same region, rather closely but is easily distinguished with a hand lens by the expanded mesosternum.

A male from San Francisco, California is 2.33 mm. long. Head subquadrate, 0.42 mm. long, 0.46 wide across eyes and 0.45 behind, with base feebly rounded and slightly impressed medially. Surface shiny, with numerous, very shallow, longitudinal strigules which anastomose occasionally; on the front these become irregular and partly transverse; these strigules visible at about 50 x magnification. Punctures moderately

sparse, very distinct but fairly small; two large, deep punctures on the occiput bear tactile setae; narrow midline without punctures. Pubescence sparse, fairly short, ca. 0.05 mm., decumbent; tactile setae of about same length, erect. Eyes small, 0.18 x 0.13 mm., not very prominent. Frontoclypeal suture distinct. Clypeus with transverse strigules, some very small punctures and sparse, decumbent pubescence. Antennae slender, gradually thickened apically. Segment I barrel-shaped, pedunculate; II thickest beyond middle, fairly stout; III-VI thickest near apex; VII subtriangular; VIII-X almost quadrate, truncate at base; XI truncate at base, tapered to a blunt point from about middle. Measurements: 10/8, 9/5, 9/5, 9/5, 9/5, 9/5, 7/7, 7/7, 7/8, 7/8, 13/8.

Prothorax widest at apical third, 0.55 mm. long, 0.20 wide at collar, 0.46 maximum and 0.36 at base, with the sides very slightly concave. Collar distinct but with groove delimiting it very shallow dorsally, so that its surface is almost continuous with the disc of the pronotum for a short distance; basal impressed line very close to base but deep and conspicuous. Surface smooth, with slight traces of strigules; punctures moderately dense, fairly large, shallow, crateriform, becoming dense in the sides of the base; the punctures bearing tactile setae are larger and deeper. Pubescence similar to that of head. Elytra subparallel, semiflattened, 1.36 mm. long, 0.56 wide at humeri, 0.74 at widest. Humeri distinct; omoplates just perceptibly elevated; suture elevated behind middle. Surface shiny, with confused, very shallow wrinkles scattered over it; punctures moderately dense, ca. 0.04 to 0.05 mm. apart, fairly small but deep. Pubescence subdecumbent, fairly long, 0.08 mm., moderately sparse; tactile setae as long as pubescence, erect, about ten in each row. Under side of thorax densely punctured and with deep longitudinal rugulae, except on edges of mesosternum, which are smooth. Abdomen finely and densely punctured. Front femora fairly slender, 0.44 x 0.15 mm.; front tibiae 0.38 mm.; hind femora 0.59 x 0.14. Pygidium 0.22 mm. wide, slightly pointed, flattened, the surface transversely microreticulate, with a few punctures and short setae near the beaded apex. Hypopygium bilobed, medially channeled and downcurved.

The front femora of the female are almost as thick as those of the male. The specimens measured range from 1.90 to 2.42 mm. long. Teneral specimens are brownish and may seem to be broader than normal dark specimens.

The aedeagus has the tegmen tapering to a point, but slightly constricted laterally near the apex, so that it is higher than wide in this region. The internal sac bears a rather large patch of spines and the gonopore armature is apparently bilaterally symmetrical and slightly hooked; it is not strongly sclerotized.

Specimens from the following localities have been examined.

UNITED STATES.—*California:* Monterey; Murphys, Calaveras Co.; Pt. San Pedro, San Mateo Co.; San Francisco and vicinity; Duncan Mills, Sonoma Co.; Ft. Baker, Marin Co.; Lodi, San Joaquin Co.; Lassen National Forest; Green Point, Humboldt Co. *Oregon:* Josephine Co.; McMinnville; Baker, Baker Co.; Siltcoos L., Yamhill Co.; Portland; Klamath Falls; French Glen; Bly Mts.; Tygh Valley; Curry Co. *Washington:* Seattle; Olympia; Coupeville; College Place; Upper Grand Coulee; Sequim; Whidby Isl.; Yakima.

CANADA.—*British Columbia:* Creston.

Apparently most abundant in early summer. Some of the samples were taken at light.

Vacusus aztecus, new species

(Fig. 4)

Shiny, uniformly piceous, tibiae and basal antennal segments paler. Prothorax slightly expanded anteriorly, as in *nigritulus* and *formicetorum*. Pubescence sparse and fine but cinereous and contrasting with the background when seen under magnification.

Holotype ♂: length 2.01 mm. Head almost evenly rounded behind eyes, smooth and shiny, with scattered fine but distinct punctures, those bearing tactile setae no deeper than others. Length 0.41 mm., width across eyes 0.41, just behind eyes 0.40. Pubescence very fine, sparse, closely appressed. Eyes small, 0.13 x 0.12 mm. Antennae rather stout throughout; segment I barrel-shaped, short-pedunculate, II-V shiny, conical, gradually wider at apex from II-V, VI-X becoming more truncate at base, distinctly punctulate, XI truncate at base and bluntly pointed. Measurements: 10/5, 8/4, 8/4, 8/5, 8/5, 7/5, 8/6, 6/6, 6/8, 6/9, 13/8.

Prothorax with sculpture and pubescence like head, with anterior portion slightly explanate and just perceptibly tuberculate at base of tactile setae; length 0.50 mm., width at collar 0.17, at widest 0.44, and 0.31 across base. Disc semiflattened; collar and basal impressed line distinct. Elytra subparallel, 1.10 mm. long, 0.46 wide at humeri and 0.60 maximum, with distinct humeri and a feeble transverse impression. Punctures moderately deep and distinct, ca. 0.04 mm. apart, with intervals smooth and shiny; pubescence sparse, appressed, straight, ca. 0.06 mm. long; tactile setae erect and of same length. Mesosternum shiny, with deep punctures except along the broad margins; marginal setae fine and just barely surpassing lateral margins of mesepisterna. Abdomen finely punctulate. Male pygidium ogival, 0.26 mm. wide, finely microreticulate; hypopygium of two deeply channeled downcurved lobes. Front femora 0.41 x 0.14 mm., front tibiae 0.32, hind femora 0.46 x 0.13.

Aedeagus with a slender tegmen, just perceptibly expanded laterally at the apex. Internal sac with some spines and gonopore armature consisting of a pair of small, closely approximated, blunt hooks.

The female has the front femora very similar to those of the male. The size of the specimens in the type series is almost uniform.

Holotype: ♂, Toluca, Est. *Mexico*, MEXICO, VIII, Wickham. Allotype: ♀, eutopotypical. Paratypes: 1♂, 2 ♀, eutopotypical. 1♀, 35 km. N.E. of Zimapan, *Hidalgo*, MEXICO, 7250′, VI-1-1948, F. Werner & W. Nutting, taken on surface of soil in daytime. The holotype and allotype are in the Museum of Comparative Zoology, Harvard University; the paratypes are in the collections of the M.C.Z. and the author.

Vacusus villosus, new species

(Fig. 5)

Ferrugineous, shiny, moderately densely clothed with long, curved, decumbent pubescence. Prothorax just slightly explanate anteriorly and with an indication of a tubercle at the base of the tactile setae of its widest place.

Holotype ♂ 2.09 mm. long. Head truncate but with temporal angles rounded, 0.38 mm. long, 0.42 wide across eyes, 0.40 behind. Surface smooth, shiny, with fine but distinct scattered punctures, those bearing tactile setae no deeper than the rest; pubescence sparse, decumbent. Eyes prominent, 0.13 x 0.08 mm. Antennae with last four or five segments thickened; segment I barrel-shaped, only slightly pedunculate, II-VI widest at apex, VII-XI truncate at base, VIII-X also truncate at apex, XI very stout and close to X, bluntly pointed; whole antenna with rather noticeable erect setae. Measurements: 8/5, 5/4, 6/4, 5/4, 5/4, 5/4, 5/5, 5/6, 5/7, 5/8, 10/8.

Prothorax 0.49 mm. long, 0.15 wide at collar, 0.44 maximum, and 0.31 across base. Surface similar to that of head but punctures slightly larger, and very sharply defined; pubescence slightly denser. Collar and basal impressed line distinct. Elytra 1.22 mm. long, 0.51 wide at humeri and 0.64 maximum, widest near middle, with distinct humeri and no trace of transverse impression. Surface shiny, moderately densely punctured, the punctures not so well defined as on pronotum; pubescence curved, subdecumbent, ca. 0.09 mm. long; tactile setae erect, ca. 0.10 mm. long. Punctures ca. 0.04 mm. apart. Mesosternum with a very few deep punctures, fringed with long setae. Metepisterna smooth, shiny, with an almost straight row of deep punctures down the middle. Abdomen finely punctured. Pygidium broadly ogival, 0.23 mm. wide, largely hidden by the preceding tergum; hypopygium of two channeled, downcurved lobes, but rather small. Front femora moderately stout, 0.38 x 0.13 mm., front tibiae 0.32 mm., hind femora 0.41 x 0.13. Legs of female almost as in male.

Aedeagus with tegmen slender, tapering, upcurved at apex and with a pair of small teeth near apex. Internal sac apparently without spines over most of its length. Gonopore armature complex, consisting of a dorsal sclerotized structure, with a crown of small spines or wrinkles just above it on the sac, and paired ventral patches of small spines in transverse rows, of which one set has been removed in the drawing for clarity.

Holotype: ♂, 30 km. N. of Jacala, Hidalgo, MEXICO (Rt. 1, km. 311), 4860′, cultivated area, VI-1-1948, F. Werner & W. Nutting. Paratypes: 1♀, eutopotypical; 1♂ Tampico, MEXICO, 14-12, E. A. Schwarz. Holotype in the M.C.Z., paratypes in the U. S. N. M. and the collection of the author.

Vacusus apicicornis (LaFerté), new combination

(Fig. 6)

Anthicus apicicornis LaFerté, 1848, Monogr. *Anthicus*: 220–1. (Type locality: Brazil; or Prov. Cumaná, Colombia, now in Venezuela.)

Anthicus plaumanni Heberdey, 1938, Rev. Ent. 8: 256. (Type locality: Nova Teutonia, Santa Catarina, Brazil.) NEW SYNONYMY.

This is a much more densely punctured species than any of the other *Vacusus* with dark patterns on the elytra. Ferrugineous, the legs and elytra paler, the latter with a narrow, slightly antemedian transverse band and a slightly oblique subapical band brown to black; finely appressed-pubescent. Last segment of antennae pale. Mesosternum without a fringe of setae but mesepisterna with conspicuous fringes along their free edges.

A male from Nova Teutonia, Brazil is 2.47 mm. long. Head subtruncate, 0.47 mm. long, 0.52 wide across eyes and 0.46 behind, with rather large, prominent eyes, 0.20 x 0.15 mm. Surface shiny, but with very feeble, fine, anastomosing longitudinal strigules; punctures moderately dense, very sharply defined but quite small, those bearing tactile setae slightly larger; pubescence very short, fine and closely appressed, almost invisible. Frontoclypeal suture almost obliterated, the clypeus small and almost continuous with plane of front. Antennae with last five segments thickened; I barrel-shaped, pedunculate; II subtriangular; III-VI thickest near apex but not truncate at base; VII-XI progressively more truncate at base; XI tapered from near base; I, II and XI paler than rest. Measurements: 10/6, 8/5, 10/5, 8/5, 8/6, 8/7, 8/8, 8/9, 8/10, 8/10, 13/10.

Prothorax 0.55 mm. long, 0.18 wide at the very short collar, 0.49 at widest and 0.38 at base, slightly expanded at the sides anteriorly and with just a trace of lateral tubercles at the base of the tactile setae. Surface more densely punctured than head, the well-defined but quite small punctures separated by about their diameters; pubescence very fine and appressed. Elytra 1.45 mm. long, 0.64 wide at the distinct humeri and 0.83 at widest, near middle, with feebly developed omoplates and transverse impression. Suture and a ridge extending obliquely posteromedially from humeri feebly elevated. Punctures small, very well defined, their sides almost elevated over the flat, smooth surface, ca. 0.03 mm. apart. Pubescence fine, ca. 0.05 mm. long, closely appressed; tactile setae ca. 0.06 mm. long and erect. Underside of thorax with fine longitudinal strigules and small punctures. Mesosternum apparently without fringe but that on mesepisterna of setae ca. 0.10 mm. long, slightly upcurved and partly visible from above. Abdomen finely punctulate; segment I longitudinally

strigulate on the sides. Male pygidium ogival, flat, almost smooth, the edge slightly upturned; the segment before it truncate. Hypopygium of two channeled, downturned lobes, quite small and capable of being retracted, with relatively long setae. Front femora 0.49 x 0.15 mm.; front tibiae 0.41; hind femora 0.59 x 0.14.

The legs of the female are almost the same as those of the male. Some specimens are darker, with the median and posterior elytral bands connected along the suture and sometimes with a dark area at the base of the elytra. The larger specimens are more heavily sculptured, especially on the head. The length of the specimens measured varies from 2.25 to 2.48 mm.

The aedeagus has the tegmen moderately slender, slightly downcurved at the apex and with a pair of small spines dorsolaterally near the apex. The gonopore armature is simple, consisting of a slightly modified tube. There seems to be no development of spines on the internal sac.

Specimens from the following localities have been examined. ARGENTINA.—*Misiones:* Bemberg, Iguazú, and Cataratas de Iguazú, Jan.-March 1945 (Hayward, Willink, and Golbach.). BRAZIL.—*Sta Catarina:* Blumenau, Nova Teutonia, 3-500 m., (F. Plaumann). TRINIDAD.—Port-of-Spain (Thaxter). COLOMBIA.—One, N.G., in Bowditch Coll. M.C.Z.

Vacusus vulgaris, new species

(Fig. 7)

Shiny, very sparsely pubescent; rufescent with head darker and elytra dark beyond basal third, except where a pale strip invades along the suture, the dark area surrounding a conspicuous luteous spot on each elytron, in the apical third. This species is superficially similar to *vicinus* but that species rarely has the pale markings of the apical third of the elytra enclosed on each elytron.

Holotype male 2.56 mm. long. Head fairly narrow, 0.49 mm. long, 0.51 wide across eyes, 0.46 behind, subtruncate, with prominent eyes, 0.19 x 0.15 mm. Surface smooth, shiny, with scattered fine punctures, those bearing tactile setae slightly larger; pubescence very fine and closely appressed, not conspicuous. Frontoclypeal suture distinct. Antennae fairly slender, gradually thicker apically; segment I almost tubular, II-X broadest near apex, becoming progressively thicker but not truncate at base, XI subparallel, blunt. Measurements: 10/6, 8/5, 8/5, 10/5, 9/5, 9/5, 9/6, 9/7, 9/8, 8/8, 13/8.

Prothorax much as in *vicinus*, 0.59 mm. long, 0.18 wide at collar, 0.46 maximum and 0.36 across base, widest at apical third but not at all explanate, the anterior portion almost evenly rounded and the sides behind slightly convex. The surface is almost like that of head but the punctures that bear the tactile setae are larger. The collar is very distinctly set off and the basal impressed line is also distinct. Elytra subparallel, 1.48 mm. long, 0.61 wide across humeri and 0.86 at widest, with distinct humeri and a very feeble transverse impression, shiny but much more deeply punctured and conspicuously pubescent than the forebody. Punctures ca. 0.04 mm. apart, deep; pubescence decumbent, slightly curved, the tips touching the surface, ca. 0.06 mm. long; tactile setae ca. 0.05 mm. long, suberect, but not very conspicuous. Mesosternum moderately densely punctured except on margins, with a very fine fringe of setae, appressed to mesepisterna. Abdomen finely punctulate. Male pygidium ogival, microreticulate, usually concealed by segment before it, which is also ogival. Hypopygium moderately large, lobed, downcurved, capable of concealment. Front femora 0.51 x 0.20 mm.; front tibiae 0.41 mm.; hind femora 0.64 x 0.17.

The female femora are not so stout as those of the male. The subapical pale spots on the elytra fuse medially in some specimens, which are also somewhat pale otherwise. In some specimens the head is almost evenly rounded behind the eyes. The length of the measured specimens varies from 2.38 to 2.82 mm.

The aedeagus has the tegmen expanded laterally and then abruptly constricted before the apex. The gonopore armature is simple, consisting of a sclerotized tube, apparently with a basal apodeme. The internal sac lacks spines and is much swollen in the specimen examined.

Holotype: ♂, Bemberg, Misiones, ARGENTINA, 12.29.I.1945 (Hayward, Willink, Golbach). Allotype: ♀, eutopotypical. Paratypes: 397, from the following localities. ARGENTINA.—*Buenos Aires:* Buenos Aires; La Plata; Isla Martín García; Tigre; Flores. *Mendoza. Entre Ríos:* Concordia; La Paz. *Córdoba:* Córdoba. *Misiones:* Bemberg; Iguazú; Cataratas de Iguazú. *Chaco:* Charate; Colonia Benítez. *Formosa:* Pirané; Mojón de Fierro. *Tucumán:* Tucumán; Parque Aconquija. *Salta. Jujuy.* URUGUAY.—Montevideo; Cerro Largo, Braile Muerto. BRAZIL.—*Mato Grosso:* Cáceres. BOLIVIA.—Cochabamba; La Paz; Obrajes; Oruoru; Quime; Hurmiri.

The holotype, allotype, and many paratypes are in the collection of the Instituto Miguel Lillo, Tucumán, Argentina. Other paratypes are in the collections of the Museo de La Plata, Museum of Comparative Zoology, U. S. National Museum, British Museum (Natural History), Mus. G. Frey, California Academy of Sciences, K. S. Hagen, and the author.

Vacusus chilensis (Solier), new combination

(Fig. 8)

Anthicus chilensis Solier, 1851, in Gay, Historia física y política de Chile 5: 277 (not seen). Fairmaire and Germain, 1863, Ann. Soc. Ent. France (4) 3: 245. (Type locality: presumably Chile.)

Generally similar to *vulgaris* but with an oval head and longer, more erect pubescence and tactile setae on the elytra. Color pattern similar to that of that species.

A male from Concon, Chile is 2.44 mm. long. Head oval, 0.49 mm. long, 0.46 wide across eyes and 0.44

behind, almost evenly rounded behind eyes, which are not very prominent and 0.15 x 0.12 mm. Surface smooth, shiny, with scattered fine punctures and appressed, moderately long pubescence. Antennae similar to those of *vulgaris*; measurements: 12/6, 8/5, 9/5, 9/5, 8/5, 8/5, 8/5, 9/5, 8/6, 8/6, 8/6, 12/6. Prothorax also similar to that of *vulgaris* but with longer appressed pubescence, 0.59 mm. long, 0.19 wide at collar, 0.46 maximum and 0.36 across base. Elytra rather narrow, widest near middle and tapering behind, 1.36 mm. long, 0.59 wide at humeri and 0.72 at widest. Humeri prominent and transverse impression poorly developed. Pubescence ca. 0.08 mm. long, straight, bristling, at about 30° angle. Tactile setae erect, ca. 0.09 mm. long, very noticeable. Underside like *vulgaris* but with pubescence somewhat longer. Front femora 0.46 x 0.18 mm.; front tibiae 0.44; hind femora 0.59 x 0.17.

There is little variation in the few specimens available for study. The aedeagus is similar to that of *vulgaris* but has the apex broader and not so strikingly dilated as in that species. The internal sac, at least in the specimen examined, is not so inflated. The gonopore armature is similar but seems to lack an apodeme.

Specimens have been examined only from CHILE: 10 mi. N. of Concon, XII-16-1950 (Ross and Michlebacher); and simply "Chile" in the Bowditch and Bolster collections, M.C.Z.

Vacusus antennatus, new species

(Fig. 9)

Generally similar to *vulgaris* but more slender, with the head evenly rounded behind eyes, the antennae longer and the elytral pubescence suberect but short and inconspicuous. The head and elytral markings are very dark in the available specimens.

The holotype male is 2.67 mm. long. Head oval, 0.52 mm. long, 0.52 wide across eyes and 0.46 behind, evenly rounded behind eyes, which are moderately prominent and 0.20 x 0.15 mm. Surface smooth, shiny, with scattered very fine punctures and inconspicuous appressed pubescence. Frontoclypeal suture distinct. Antennae unusually long, the basal three or four and the last segment paler than the rest. Segments somewhat disjointed, not truncate at base; XI bluntly pointed. Measurements: 13/6, 12/5, 13/5, 12/5, 12/6, 12/7, 12/8, 12/8, 10/8, 10/8, 15/9.

Prothorax of same general shape as in *vulgaris* but narrower and not at all flattened on disc, 0.69 mm. long, 0.19 wide at collar, 0.46 maximum, 0.33 in subbasal constriction and 0.36 across base. Surface almost as on head but with punctures slightly larger and the ones bearing tactile setae conspicuously larger. Elytra widest just beyond middle, 1.46 mm. long, 0.61 wide across humeri and 0.79 at widest, with distinct humeri and a feeble transverse impression. Surface shiny, with deep punctures ca. 0.05 mm. apart; pubescence short, suberect, at ca. 45° angle, ca. 0.04 mm. long but slightly longer near apex; tactile setae only slightly more erect than pubescence, ca. 0.08 mm. long. The two available specimens have been somewhat rubbed and may have the tactile setae pushed over more than is normal. Underside almost as in *vulgaris*. Front femora 0.56 x 0.20 mm.; front tibiae 0.49 mm.; hind femora not in a position for measurement.

The aedeagus has the tegmen almost as in *vulgaris*, but has the subapical lateral dilation distinctly pointed posteriorly. The internal sac and the gonopore armature are probably the same.

Holotype: ♂, Ingeniero Juárez, Formosa, ARGENTINA, Jan. 7, 1949, R. Golbach, in Instituto Miguel Lillo. Paratype: 1 ♂, Tucumán, ARGENTINA, Apr. 24, 1955, R. Goldbach, in the collection of the author.

Vacusus fulvonotatus (LaFerté), new combination

Anthicus fulvonotatus LaFerté, 1848, Monogr. *Anthicus*: 156–7. (Type locality: Colombia.)

No specimens of this species have been available for detailed study. The single type specimen, in the LaFerté collection in the Muséum National d'Histoire Naturelle in Paris, has been examined, however. It is a female and has the head and prothorax very narrow for members of this genus. The head is evenly rounded behind the eyes and at least half its length lies behind the eyes. The prothorax lacks the deep punctures that bear the tactile setae in *vicinus* but is otherwise similar in sculpture. The elytral pubescence is very much as in *vicinus*. The femora are stout, even though the specimen is a female. This species must be similar to *antennatus* but a specimen of that species was compared with the present one in Paris and deemed to be different. The two differ at least in elytral pubescence, and probably in the degree of narrowness of the forebody.

Vacusus peruvianus (Pic), new combination

Anthicus peruvianus Pic, 1902, Échange 18: 31. (Type locality: Prov. Tumbez, N. Peru (Grau), from data on type specimen in Pic collection in Paris.)

This species is very distinct and may not actually belong in the assemblage of species related to *vulgaris*. The specimens available for study are all females and the aedeagus cannot therefore be compared with that of other species. The species bears a general resemblance to *vulgaris* but has the forebody all rufous, not with darkened head, and has the forebody much more deeply and distinctly punctured. The elytral pubescence is short and appressed but the tactile setae are long and erect.

A female from Piura, Peru is 2.82 mm. long. Head truncate but with rounded tempora, 0.49 mm. long, 0.54 wide across eyes and 0.47 behind; with prominent eyes, which are 0.20 x 0.13 mm. The surface is shiny, with scattered but very sharply defined and moderately deep punctures, those bearing tactile setae almost the same as the rest; pubescence very sparse, short, appressed; tactile setae erect, ca.

0.08 mm. long. Antennal segments VIII-XI truncate basally, IX-X almost quadrate; thickened segments circular in cross-section. Measurements: 13/6, 8/5, 9/5, 9/5, 9/5, 8/5, 8/8, 12/9, 8/6, 8/7, 8/8, 8/8, 12/9.

Prothorax like that of *vulgaris* but with punctures and pubescence like head, 0.59 mm. long, 0.20 wide at collar, 0.51 maximum and 0.38 across base. Elytra broadest near middle, 1.74 mm. long, 0.72 wide across humeri and 0.79 at widest, with well defined humeri and a feeble transverse impression. Punctures small and very well defined, the edges almost vertical to the very flat surface, ca. 0.04 mm. apart; pubescence short, ca. 0.04 mm. long, appressed; tactile setae erect, ca. 0.08 mm. long. Posterior spot open laterally. Underside much as in *vulgaris* but abdomen finely microreticulate. Front femora 0.51 x 0.15 mm.; front tibiae 0.41; hind femora 0.63 x 0.15.

The three specimens examined, one of which was compared with the type, are from Piura, PERU, Oct. 29, 1941, P. A. Berry, on cotton buds, in the U.S.N.M.

Vacusus subbrunneipennis (Pic) new combination
(Fig. 10)

Anthicus vicinus var. *subbrunneipennis* Pic, 1933, Mel. exot.-ent. 61: 30. (Type locality: R. Argentine, Coll. Bruch.)

Dark rufous, the elytra darker; shiny; of general aspect of *vulgaris* but head and prothorax more deeply punctured; elytral pubescence short and decumbent, the tactile setae long and erect. Markings on elytra very ill-defined, if present at all.

A male from La Paz, Bolivia is 3.24 mm. long. Head truncate but with rounded tempora, 0.59 mm. long, 0.60 wide across eyes and 0.59 behind; eyes not very prominent, 0.23 x 0.15 mm. Surface shiny, with the punctures small but well defined and deep, those bearing tactile setae almost like the rest; pubescence very sparse, short, appressed; tactile setae long and erect. Frontoclypeal suture almost obsolete. Antennae uniformly colored, the wider segments subtriangular, not truncate at base. Measurements: 13/6, 12/5, 13/5, 12/5, 10/5, 12/5, 12/8, 12/8, 12/8, 10/8, 15/8.

Prothorax as in *vulgaris* but with deep punctures, those toward base almost as large as those on elytra and almost the same distance apart. Length 0.76 mm., width 0.27 at collar, 0.61 maximum and 0.36 across base. Elytra subparallel, 1.89 mm. long, 0.77 wide across humeri and 1.02 maximum, with well defined humeri and weak transverse impression and omoplates. Surface shiny; punctures moderately large and deep, ca. 0.05 mm. apart. Pubescence short, ca. 0.05 mm. long and closely appressed; tactile setae erect, ca. 0.09 mm. long. Mesosternum deeply punctured and microreticulate but shiny, except for the smooth, shiny margins; marginal fringe very fine and appressed. Rest of underside microreticulate, as well as rather deeply punctured. Pygidium ca. 0.38 mm. wide, ogival, flat. Hypopygial lobes rather large but only slightly downcurved. Front femora 0.59 x 0.22 mm.; front tibiae 0.51; hind femora 0.77 x 0.20.

The femora of the female are almost like those of the male. The length of the specimens examined varies very little. In most the elytra are uniformly dark, or with a very feeble indication of a color pattern in transmitted light; in one specimen the base of the elytra is paler.

The aedeagus has the tegmen evenly tapered and slightly downcurved at the apex. The gonopore armature consists of a flat sclerotized cone, with a patch of spines on each side.

Specimens have been examined from the following localities. ARGENTINA.—*Buenos Aires. Mendoza. San Luis:* Estancia Don Roberto, Lavaisse, Oct. 17, 1942, W. Wittmer (B.M.). *Rio Negro:* Rio Colorado, Dec. 1930. BOLIVIA.—La Paz and Cochabamba.

Vascusus parvus (Pic), new combination
(Fig. 11)

Anthicus parvus Pic, 1910, Rev. Mus. La Plata 17: 109. (Type locality: Prov. Buenos Aires, ARGENTINA.)
Anthicus parvus var. *atratulus* Pic, 1912, An. Mus. Nac. Buenos Aires 22: 456. (Type locality: Prov. Buenos Aires, Argentina.) NEW SYNONYMY.
Anthicus parvus var. *weiseri* Pic, 1926, Mél. exot.-ent. 47: 25. (Type locality: Argentine, Coll. Bruch.) NEW SYNONYMY.

A small, very shiny, almost glabrous species with a truncate, slightly retrosalient head. Head and usually elytra brown, prothorax and appendages pale; elytra sometimes pale across base or all pale. Elytra with short, appressed pubescence and long, erect, tactile setae.

A male from Llavalol, Argentina, is 1.79 mm. long. Head truncate, slightly retrosalient, 0.35 mm. long, 0.38 wide across eyes and 0.37 behind; eyes not very prominent, 0.12 x 0.10 mm. Frontoclypeal suture weak. Surface shiny, with scattered small punctures, those bearing tactile setae no larger than the others; pubescence short and appressed, very sparse. Antennal segments VII-X submoniliform. Measurements: 6/5, 6/4, 5/4, 5/4, 5/4, 5/4, 5/5, 5/5, 5/6, 5/6, 9/6. Last segment of maxillary palpi rather large, securiform.

Prothorax slightly flattened, 0.42 mm. long, 0.15 wide at collar, 0.37 maximum and 0.28 across base, subcordate because the collar is short and almost set into the main portion; sides almost straight behind the rounded broadest portion, which is set very far forward. Basal impressed line distinct. Surface almost like head. Elytra subparallel, 1.02 mm. long, 0.41 wide across humeri and 0.61 at widest, semiflattened. The punctures are small but distinct, ca. 0.04 mm. apart; pubescence appressed, ca. 0.04 mm. long; tactile setae erect, ca. 0.06 mm. long. Mesosternum broadly expanded, covering the mesepisterna, punctured in middle but smooth and shiny laterally, with a fringe of long, curved setae laterally and shorter, curved setae behind. Abdomen punctulate. Pygidium ogival, ca. 0.19 mm. wide, often largely

hidden by the segment before it. Hypopygial lobes short and downcurved. Front femora 0.29 x 0.12 mm.; front tibiae 0.26 and hind femora 0.38 x 0.11.

In the female the front femora are slightly more slender. There seems to be very little variation in the length of the specimens in the series at hand.

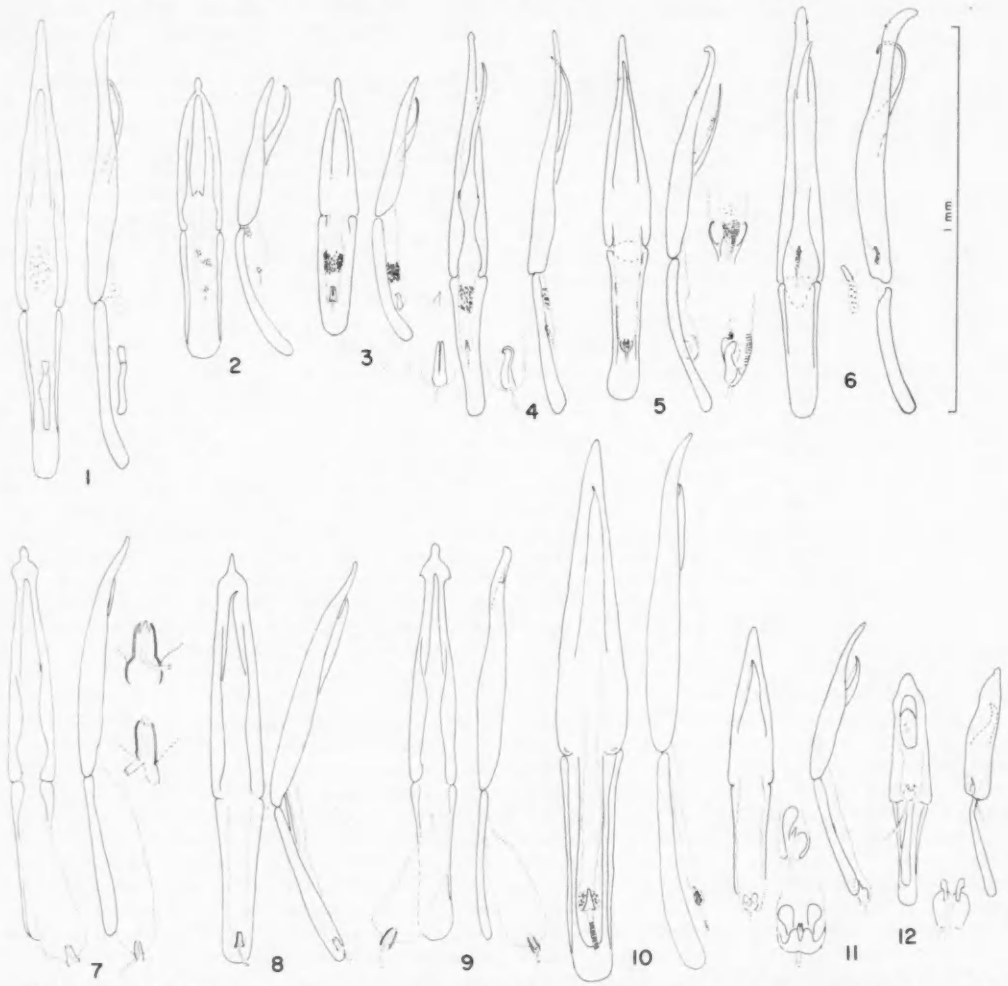
The aedeagus has the tegmen gradually tapered to a blunt point, somewhat as in some other species in the genus. The gonopore armature is complex and unique, consisting of paired ventral, dorsal and median lobes, the ductus ejaculatorius apparently connecting to the median pair.

Specimens from the following localities have been examined. ARGENTINA.—*Buenos Aires:* Flores; Tigre; Llavalol (R. Thaxter). *La Pampa:* General Pico. *Misiones:* Puerto Aguirre. URUGUAY.— Maldonado (C. Darwin). BRAZIL.—*Mato Grosso:* Cáceres. One of the specimens from Buenos Aires Province is labeled as having been taken in a nest of *Atta* sp.

Vacusus holoxanthus (Fairmaire and Germain) new combination

Anthicus holoxanthus Fairmaire and Germain, 1860, Coleoptera chilensia, Paris, p. 3 (not seen); 1863, Ann. Soc. Ent. France (4) 3: 249. (Type locality: Santiago?, Chile.)

This is a small, pale, parallel-sided species, with truncate head, quite dense punctures and short pubescence and tactile setae.



FIGS. 1-12.. Aedeagi in ventral and left lateral view as they lie in the abdomen, some with insert figures to show details of the gonopore armature. 1. *V. vicinus* (Laf.). 2. *V. formicetorum* (Wasm.). 3. *V. nigritulus* (Lec.). 4. *V. aztecus*, sp. n. 5. *V. villosus*, sp. n. 6. *V. apicicornis* (Laf.). 7. *V. vulgaris*, sp. n. 8. *V. chilensis* (Solier). 9. *V. antennatus*, sp. n. 10. *V. subbrunneipennis* (Pic.). 11. *V. parvus* (Pic.). 12. *V. jamaicanus* sp. n.

A female specimen in the M.C.Z. labeled "Chi." is 2.12 mm. long. Head truncate and slightly retrosalient, 0.44 mm. long, 0.41 wide across eyes and 0.40 behind; eyes not very prominent, 0.18 x 0.15 mm. Frontoclypeal suture distinct. Surface with moderately dense (ca. 0.03 mm. apart), fairly small and shallow but well-defined punctures, the intervals smooth and shiny. Pubescence decumbent and fine. Antennal segments VII-X submoniliform, XI tapering from basal third. Measurements: 8/5, 5/4, 6/4, 5/4, 5/4, 5/4, 5/5, 5/5, 5/5, 5/5, 10/5.

Prothorax widest at apical fourth, very straight-sided from there to base, 0.50 mm. long, 0.18 wide at collar, 0.41 maximum and 0.31 across base; collar and basal impressed line distinct. Surface almost like that of head, with punctures and pubescence slightly denser. Elytra parallel, 1.18 mm. long, 0.49 wide at humeri and 0.61 maximum, with distinct humeri but no transverse impression; suture not at all elevated, the curve of the elytra continuous across it in a very unusual way. Punctures fairly small and shallow, ca. 0.03 mm. apart; pubescence decumbent, ca. 0.05 mm. long; tactile setae almost erect, slightly curved, but only 0.03 mm. long. Mesosternum with sparse, deep punctures, except on a narrow, smooth margin, fringed with fine setae appressed to mesepisterna. Abdomen finely punctulate. Front femora 0.38 x 0.10 mm.; front tibiae 0.27; hind femora 0.46 x 0.10.

There is little variation in the four female specimens available for study. These consist of two specimens in the Museum of Comparative Zoology from CHILE, without exact locality, and two in the U. S. National Museum from ARGENTINA: *Salta:* Tartagal and General Ballivian, collected by G. L. Harrington.

Vacusus jamaicanus, new species

(Fig. 12)

This is a slender, luteous species with truncate head, narrow prothorax, moderately heavy antennae, prominent eyes and moderately dense punctures. The pubescence is fine, appressed, and the tactile setae are short and almost indistinguishable.

Holotype male: length 1.79 mm. Head truncate, 0.33 mm. long, 0.38 wide across eyes and 0.35 behind, with prominent eyes 0.17 x 0.13 mm. Frontoclypeal suture almost obsolete. Surface shiny, with moderately dense but fine, well defined punctures, which are absent on midline of frons. Pubescence fine and appressed. The thickest antennal segments are subquadrate. Measurements: 8/5, 5/3, 5/3, 5/4, 5/4, 5/4, 5/5, 5/5, 5/5, 5/5, 9/5.

Prothorax widest at apical fourth, with sides straight behind, 0.46 mm. long, 0.17 wide at collar, 0.35 maximum and 0.28 across base. Surface much like head; disc not flattened; collar and basal impressed line distinct. Elytra subparallel, 1.00 mm. long, 0.44 wide at humeri and 0.54 maximum, with distinct humeri and obsolete transverse impression. The punctures are moderately deep and ca. 0.03 mm. apart; the intervals are shiny but slightly irregular on the surface. Pubescence ca. 0.03 mm. long, closely appressed; tactile setae ca. 0.02 mm. long, erect but inconspicuous. Mesosternum shiny but with a few fine punctures; fringe closely appressed to the mesepisterna, which themselves have short fringes. Pygidium rounded apically, not covered by the segment before it; hypopygium small. Front femora 0.33 x 0.12 mm.; front tibiae 0.28; hind femora 0.42 x 0.12.

The female femora are almost as stout as those of the male. The specimens measured range from 1.79 to 2.00 mm. in length and all are pale in hue.

The aedeagus has the tegmen unusually stout for members of this genus, and constricted near the apex. The opening which surrounds the penis seems to be completely sclerotized all around; this condition is very unusual in the family Anthicidae. The internal sac seems to lack spines. The gonopore armature consists of a small sclerotized plate, with a pair of blunt hooks attached to its apex.

Holotype ♂, allotype ♀, and 8 paratypes (2♂, 6♀): JAMAICA, Spa[nish] Town, II-2-1937, Sta. 377, flying at dusk (Chapin and Blackwelder). Other paratypes, all collected by Chapin and Blackwelder, from JAMAICA, as follows: 1♂ Milk River, II-25-1937, Sta. 415; 6♂, 6♀, Morant Bay, II-6-1937, Sta. 391; 1♀, Gordon Tn., II-1-1937, Sta. 367. Holotype, allotype, and paratypes in the U. S. National Museum; paratypes also in the collection of the author.

Laboratory Studies on the Adult Behavior of *Conopthorus coniperda* (Schwarz) (Coleoptera: Scolytidae). II. Thigmotropic Aggregation[1]

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ABSTRACT

The white pine cone beetle (*Conophthorus coniperda* (Schwarz)) forms aggregations when confined in open arenas in the laboratory. These aggregations are stable and immobile. They appear to be symptomatic of a strong thigmotropism which leads the insect to increase its body contact. The extent of aggregation is increased by a variety of physical conditions which tend to increase overall activity and thus provide more opportunities for collisions. The seasonal change in the reaction makes it a useful model for the investigation of aggregation in general, as well as providing additional understanding of the field behavior of the insect.

The aggregation of animals may arise from a number of sources. Baerends (1959) lists local attractiveness, conspecific attraction, communication, chemical bonds and cast interdependence as causes of aggregation. It may also be modified by the sex of the individuals quite apart from sexual affinities. The effect of density on aggregation has been treated by many authors. For example, Naylor (1959) found by experimental analysis that *Tribolium confusum* DuVal is highly aggregated at low densities, becomes random at moderate densities, and underdispersed at very high densities. A similar conclusion was reached from the analysis of field sampling records by Waters and Henson (1959). Hairston (1959), during the course of community organization analysis, found that the rare species in a community are most highly aggregated. Thus, on the grounds of three different sorts of evidence, it is possible to conclude that density is closely related to the effectiveness of the aggregation producing mechanisms.

The tendency of animals to aggregate is of the greatest ecological importance both for its selective effect on the animals and because of the effect of the habit on the food plants or other substrate. Dethier (1959a) has pointed out that the numbers of individuals of any generation which become established are affected not only by the conventional sources of egg mortality but also by parental errors in searching and deposition. Searching behavior is much influenced by mutual interactions (Burnett 1958). The dispersal of larvae and their tendencies to aggregate have a major effect on the manner in which insects utilize their substrate (Dethier 1959b). If the aggregative tendency is affected by density as it seems to be in a number of cases, the capacity of an insect to exhaust its substrate may be affected by aggregative change with increasing density (Henson 1959).

The aggregation of insects on their substrate produces crowding within the aggregations. The biological effects of this are probably profound. At the very least, competition within the confines of the aggregation for food and space may take place. Long (1953) has described the effects of crowding on larvae of Lepidoptera. Profound morphological and physiological consequences, including color changes, modification of growth rates and fecundity and overall size changes, have been shown. These changes are at times different from those which would be expected on intuitive grounds. In *Pieris brassicae* L., crowding prolongs pupal development but reduces the total time from egg to adult by speeding feeding and larval development. On the other hand, in *Plusia gamma* L., crowding prolongs adult preoviposition time but reduces total time of a generation by speeding growth (Zaher and Long 1959).

The experimental analysis of aggregation has been rather restricted. Naylor (1959) investigated the behavior of *Tribolium* from a statistical viewpoint. A number of investigations have been carried out from this angle (for literature see Waters and Henson 1959), but most of them have been concerned with sampling theory rather than insect biology. Long (1955) investigated two Lepidoptera and found that the effective mechanism in the aggregation of *Pieris brassicae* was the deposition of silk trails. This mechanism is reminiscent of the more social behavior of *Malacosoma disstria,* Hübner, which is treated from rather a different aspect by Wellington (1960). In *Plusia gamma,* Long (1955) found that larval contact was the effective stimulus to aggregation. The probability of collision leading to such contact was increased by the mutual orientation to light and the attraction of the animals to a common food plant. Most insects probably aggregate by means of a variety of mechanisms. Ellis and Ashall (1957) cite mutual interaction, discontinuity of substrate, and restricted zones of temperature, radiation, and air movement as being instrumental in the aggregation of *Schistocerca gregaria* Forskål.

The investigation of aggregation has in the past followed rather a restricted number of lines. The most general approach has been the direct observation of insect activity, perhaps reinforced by observations of the substrate or measurement of physical conditions. A very powerful but unusual approach has been the direct investigation of behavior followed by the analysis of field observations (Wellington 1957). The statistical analysis of field population data has been used with limited success (Waters and Henson 1959). Here, the existence of aggregation is easy to demonstrate, but only presumptive evidence is gained as to the nature of the mechanism.

[1] Accepted for publication May 10, 1961.

An experimental laboratory analysis following the approach of Naylor (1959) has also proved powerful in the past. Probably the approach used should be selected with reference to the peculiarities of the insect and with respect to the objectives or emphasis of the investigation.

THE MEASUREMENT OF AGGREGATION

The means employed to measure aggregation depend to a considerable extent on the nature of the interest in the phenomenon. The classic approach has been to make frequency counts of animals within the boundaries of standard-size plots. The distribution of frequencies is compared with that to be expected on the basis of random placement for the observed density. In this way, underdispersion or overdispersion may be detected and its degree expressed in terms of the standard fiducial expectations. This approach has been extensively investigated by Waters and Henson (1959). In general, it was found that there are at least two components in contagion as measured by frequency distribution characteristics. First, there is the overt tendency of the animals to aggregate. Second, there is the discontinuity of the substrate, which together with the arbitrary nature of the choice of sample unit size, tends to impose an evidence of contagion which is more a statistical artifact than a biological response.

In the same investigation, Waters and Henson (1959) showed evidence that the size of the sample as well as the size of the sample unit can impose artifacts on evidences of contagion. The use of an index of diversion which is a refinement of the frequency distribution technique has been shown by Hairston (1959) to be subject to similar errors.

From the viewpoint of experimental work, the use of frequency counts is a cumbersome method. The evidence on which sample unit size is regulated in field studies (Waters and Henson 1959) is missing in the laboratory. In addition, rather large numbers of individuals are required to give sufficient counts from which to erect the required distribution.

During the past few years, plant ecologists have been increasingly interested in the spatial distribution of plants and their communities. This interest has related both to the interaction between plants and to the grouping of plants on a discontinuous substrate. On the basis of Skellam's (1952) theoretical development of distribution as related to spatial pattern, a variety of plotless techniques for plant ecology has been developed. The earliest of these depended on the use of random pairs of individuals (Shanks 1954). It was found, however, that random-pairs plots result in the underestimation of contagiously distributed species. Various transformations were proposed to deal with this limitation.

Clark and Evans (1954) used the distance between nearest neighbors as a measure of spatial relationship. This approach avoided the statistical difficulties of random pair techniques while retaining the advantage of plotless technique. The technique involved the comparison of the mean distance between nearest neighbors with that mean distance to be expected in random dispersion of the observed density. The calculation of this comparison is simple, and formulae for the estimation of errors are provided. In a later paper, Cottam and Curtis (1956) reviewed the use of the technique. Their findings indicated that the technique is efficient. Thompson (1956) extended the theory to include not only nearest neighbors but the distance to the n^{th} neighbor in populations of randomly distributed individuals.

Cottam et al. (1957) applied nearest-neighbor measurement to a series of aggregated populations. It was found that the technique provides an efficient measurement of within-clump mean area in aggregated populations.

On the basis of these findings, it was decided to use nearest-neighbor technique for the detection of aggregation within laboratory-maintained groups of *Conophthorus coniperda* (Schwarz). The technique appears to be strongest when the absolute density is known independently. Blackith (1958) has shown that when nearest-neighbor measurements must form the basis for density estimates, the errors depend on the degree of departure from random dispersion. Overdispersion increases the error directly, while underdispersion appears to have little effect. In the investigation of most nonsocial animals without territorial community structure, then, one would expect considerable error from density measurements based on nearest-neighbor determinations.

The technique employed in the present study was to photograph the experimental group of animals at the moment when a determination was required. The photographs were printed life size. Measurements of distances between nearest neighbors were made by means of dividers reading directly from the photographs. The measured individuals were marked on the photograph before the measurement was recorded. Distance between individuals was taken to the nearest half-millimeter between the closest parts of the bodies. Measurements were not taken to legs or antennae. The measurements were recorded in the form of a frequency distribution of distances by 1-millimeter classes, with insects in contact being lumped with insects less than 0.5 millimeters apart. This lumping was done because of the difficulty of defining contact. The calculation of statistics followed the notation and formulae given by Clark and Evans (1954).

OBSERVATIONS

Conophthorus coniperda (Schwarz) is a scolytid pest of white pines. The insect is widespread, but has apparently attracted little attention. The life history has been given by Godwin (1959). Some aspects of its biology have been outlined in a previous contribution to this series (Henson 1960). During the course of investigations of the responses of the insect to physical factors, it became apparent that one of the most predominant characteristics of the

animal was its thigmotropic aggregation. The effects of this reaction on temperature response have already been described (Henson 1960). This paper is a report of an investigation of the aggregation.

When adult *Conophthorus coniperda*, freshly dissected from overwintering cones, are placed in a container floored with filter paper or some other material which gives a good footing, the animals wander until they contact a solid object. When a small container is used, in most cases the object will be the walls of the container. If this be the case, the insects will follow the walls during further movement. When glass petri dishes are used, the slight rounding at the junction of the walls and floor of the container is enough to prevent the animals from following the walls. If two animals collide, they stop moving in almost all cases and remain together in contact. If a third animal encounters the pair, it will similarly remain in contact. In this way, large aggregations are formed. These persist for hours if the animals are undisturbed and unstressed. The behavior, and one of its primary implications, has been described elsewhere (Henson 1960).

Single insects in an open arena will tend to locate themselves near the walls. This is frequently an inevitable outcome of the insect's predominantly straight path in an enclosed space and in the absence of gradients or directional light sources. Since petri dishes with their rounded walls did not cause permanent fixing of the insects, the tendency for insects to be near the walls was accepted in the experiments. Single insects were placed in petri dishes floored with filter paper. The dishes were lighted from a diffuse source overhead. The animals were protected from air movement and all other detectable directional stimuli. Their tracks were recorded with time checks to permit rate determinations. From these tracks, the number of changes in direction per unit time could be calculated. The experiments were repeated with two insects in the arena at the same time. The rate of change of movement and direction was plotted as a function of the instantaneous distance between the two insects. In this way, it was found that the insects did not react to each other except perhaps at very short ranges. At ranges of less than 4 millimeters, the animals turned toward each other and formed an aggregation. It was not possible to show any interaction at greater range. Because the recognition range was so short, the probability that two insects would form an aggregation appeared to be only very slightly different from the probability of collision. This finding suggests a relationship between speed of movement and aggregation which was demonstrated in an earlier contribution (Henson 1960). The relationship between density and aggregation will be treated later in this paper.

The behavior of individual insects toward grains of rice, either natural or black in color, short lengths of wire, and a variety of other objects about the same size and shape as themselves could not be separated from their behavior toward dead or tethered insects. There was no evidence of chemical attraction. Dead animals attracted as many live insects as tethered living animals. Extraction with boiling alcohol, boiling xylene, hot water, and a variety of other agents did not reduce the attractiveness of dead tethered insects or yield an extract which could be shown to be active in an olefactometer. Nor was the sex of attracted animals related to that of tethered animals. Pairs of aggregated animals taken from culture were random with respect to sex. In addition, individuals at any opportunity moved under filter paper scraps or into other shelters which would admit them. The amount or direction of the light had no effect on these reactions even in the case of strongly photopositive animals.

These findings indicate that the laboratory agregation of *Conophthorus coniperda* is not a case of any of the sorts listed by Baerends (1959), with the possible exception of local attractiveness. The locale represented by an insect, whether dead or alive, extracted or not, a grain of rice, or any other object of suitable size, is attractive. This interpretation of the classification of Baerends is an unwarranted extension. I think that the aggregation of *Conophthorus* must be described as a laboratory artifact which arises from a very strong thigmotropism. It is interesting to note that the reaction can be seen in all adults except those which have emerged from their cones and are undergoing dispersal. It appears strongest, however, in animals taken from overwintering cones, and the experimental work was confined to such animals.

In the literature on thigmotropic responses, there appears to be no record of attempts to provide maximal body contact by artificial means and thereby produce the semblence of an aggregation. Such a manipulation, if possible, would provide the most convenient approach to a physiological investigation of the mechanisms involved in thigmotropism.

Attempts were made to fix insects by placing them in contact with filter paper, dead insects, dummies, and living insects. All these attempts failed. The free, moving insects moved away from all objects touched against them even when they were taken from stable aggregations, presumably in a state where they were inclined to aggregate.

After groups of insects had been held together in containers without a flooring of filter paper or other material to give good footing, a number of the stored animals seemed unable to move. Examination revealed that they were suffering from extensive wounding of the legs and in some cases of the antennae. A rough measure of the extent of this damage could be made simply by counting the number of amputations on a group of beetles. A rather constant damage amounting to 0.6 amputations of tarsal joints per animal was found in the overwintering population. All attempts to form aggregations by mechanical juxtaposition of the animals by various confinement techniques resulted in mutual wounding.

Interestingly enough, the extent of the wounding after a standard period of confinement at a standard density was very constant. A critical density had to be maintained or the animals within their confinement simply avoided each other or formed normal aggregations. Above this critical density, however, they grappled and removed each other's tarsi. Providing a firm footing increased the density which could be maintained without injury.

Insects confined in small containers could be exposed conveniently to a variety of experimental conditions. A count of missing tarsal joints would then yield a rough indication of the overall level of activity induced by the experimental conditions.

The importance of these observations in the present context is that they illustrate the curious fact that though the animals are very strongly thigmotropic, they resist attempts to increase their degree of body contact. Two insects will resist all attempts to place them in contact with each other and then, on release, they may well form a stable aggregation. Apparently there is some kinaesthetic effect required in the formation of an aggregation, or the areas of contact on the body wall are very critical, or the experimenter's handling techniques were such as to evoke an avoidance on the part of directly manipulated insects.

The finding that the insects do not respond to mechanical crowding as they do to physical contact resulting from their own activity is particularly important. It suggests that the fundamental behavior involved in aggregation of *Conophthorus* has little to do with the mere fact of mutual contact between insects. This confirms the earlier impression that the aggregations seen in laboratory studies are artifacts in the sense that they result from the accidental satisfaction of the kinaesthetic drive by contact with other animals. Any other comparable physical situation serves as well.

This being the case, it is of interest to examine the effects of a series of physical factors on thigmotropic aggregation. In this way, some of the characteristics of the thigmotropism itself can be illustrated. Again, there is an interest in the effects of this thigmotropism in the modification of the direct response of the insect to various physical factors. In later contributions to this series, I propose to report the effects of a series of physical factors on the behavior of insects tested singly and insects tested during their normal dispersive phase when the thigmotropism is lost. For the present study, the characteristics of the thigmotropic response and its possible selective significance are of greatest interest.

The Rate of Aggregation

In order to establish the rate at which aggregation proceeds, a large series of insects was scattered in each of three petri dishes and their aggregation measured at intervals. The results in the form of mean values of R (Clark and Evans 1954) are given in table 1.

The findings reported in table 1 suggest that aggregation is very rapid during the first few minutes and that it becomes steady with further time. It should be mentioned in connection with this initial table that an "R" value of 1 indicates random association. An "R" value of 0.0 indicates complete aggregation, with each individual in contact with its nearest neighbor and a mean nearest-neighbor measurement of 0.

Table 1.—Rate of aggregation of *Conophthorus coniperda* exposed at room temperature and humidity[a].

Time (min.)	R	S_{re}
0	0.683	0.177
0.5	0.657	0.178
1.0	0.548	0.178
2	0.370	0.173
5	0.463	0.199
10	0.380	0.187
30	0.364	0.201
60	0.336	0.219

[a] 200 animals per run.

Because the aggregation, at least at the high density tested here, was almost complete in a short time, further testing was standardized with runs of 30 minutes, unless otherwise specified.

The Limit of Aggregation

It will be noted in the experiments reported in table 1 that, in spite of very high densities of insects and a considerable length of time being allowed, the ultimate extent of aggregation still fell considerably short of complete involvement of all individuals. As was already established, the reaction is a result of thigmotropism, perhaps very slightly reinforced by visual stimuli. An attempt was made to estimate the proportion of the stock culture of insects which, under standard circumstances of maximum opportunity, could be expected to aggregate.

Nine-cm. petri dishes floored with filter paper were provided. Half-sheets of filter paper were cut into pieces about 1 sq. cm. in area. Each of these pieces was bent slightly so it would not lie flat. The floors of each petri plate were scattered with bent paper fragments, making sure that there was no overlapping of the fragments. In this way, an arena was presented in which half the area was under filter paper shelter. Groups of 20 beetles from stock were put in each dish and left for 30 minutes. The number visible was counted as being not thigmotropic. The assumption was that all insects would have had the chance to move under cover in the time allowed. 82% ± 3.4% of the insects tested in 10 such runs moved under the shelter provided. As reported in a previous contribution, a variable proportion of the insects is exceedingly emaciated. In the present stock about 25% suffered from this condition. The failure to aggregate on the part of between 15% and 20% of the stock may be related to starvation. The incapacitated animals

would, of course, serve as passive objects in aggregation, but would not themselves initiate an aggregation.

Visual Stimuli

An early finding of the study was that insects would turn toward each other and form aggregations when within very short range. In order to test the overall effect of this, the only known nonthigmotropic component, on the extent of aggregation, duplicate runs were made with 50 insects per run in light and in absolute dark. The experiment was replicated.

The results summarized in table 2 show that the reaction of the animals is both a visual reaction and a thigmotropism. Since the response to cover reported in the previous experiments certainly involves more than 80% of the stock population, one is led to suspect that the effect demonstrated for light may involve a phototaxis on the part of the animals. In other words, they may aggregate more in light, not because they see other individuals and move toward them, but because the light stimulates them to move and this movement increases the probability of collision.

Table 2.—The effect of light on the extent of aggregation in *Conophthorus coniperda*[a].

	Run 1	Run 2
Room daylight	0.229	0.240
Dark	0.361	0.448

[a] Room temperature and humidity; 50 insects per run. Values given are for R. Probability that lighting conditions were effective is greater than 99%.

In order to test the effect of the probability of collision on the extent of aggregation, means were sought in which this probability could be manipulated without physiological stress to the experimental animals. It was earlier demonstrated that when walking speed was increased by increases in temperature, there was an increased tendency to aggregate. For the present tests, a second manipulation was employed.

The Effect of Density

A series of graduated numbers of insects was exposed for 10 minutes in standard petri dishes at room temperature and humidity in room daylight. The amount of variability in aggregation, particularly at the lower densities, was very great. The experiments were replicated at all densities except those of 120 and 200 insects per container, 7 replications being used. Limitations in the amount of stock which could be provided on a single day prevented further replication. In figure 1, the mean and range in the amount of aggregation as a function of density is illustrated.

The data presented in figure 1 show that the formation of aggregations is increased by increases in density up to a point equivalent to a density of perhaps 100 insects per 9-cm. petri plate. Beyond this point, little increase in the amount of aggregation is seen. The extreme variability at the lowest density suggests the influence that one accidental collision can have on the mean distance between nearest neighbors. At such densities, a very great deal of replication would be required to give a good estimate of the mean index of aggregation.

The implication of these results is that the extent

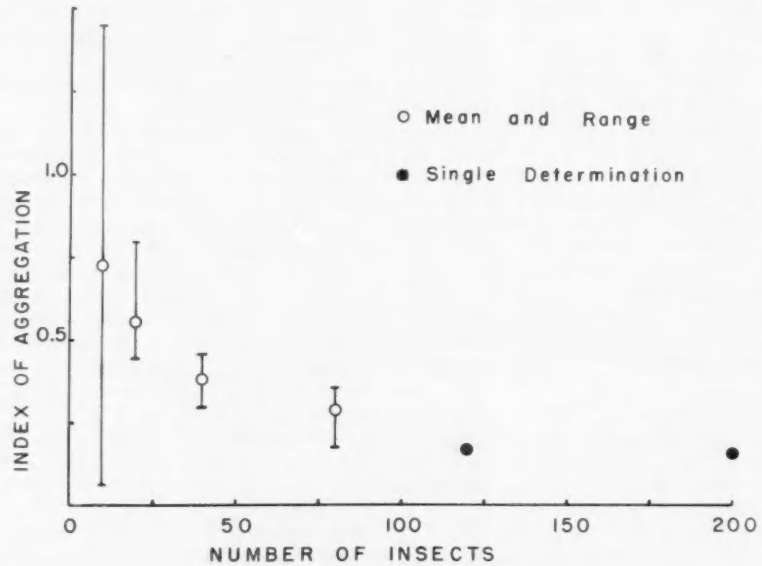

FIG. 1.—The effect of density on aggregation.

of aggregation will be increased by any manipulation which has the effect of increasing the probability of collision. In the work in temperature gradients reported previously (Henson 1960) the concentration of insects in a favored part of the gradient in effect increased density. The enhanced speed of movement in the upper temperature zones also tended to increase the probability of collision.

A more artificial method of providing an increase in density was suggested by the initial observation that insects would aggregate on models such as rice grains. Insects were placed in standard petri plates floored with filter paper, with 40 insects per container. The degree of aggregation was compared with that shown by groups of 20 insects in a container with 20 dummies made of black insulated copper wire fragments about the size of the insects. In the latter determination, the dummies were treated as insects for the purpose of analysis. Again, groups of 40 insects with 40 dummies were compared with groups of 80 insects. The entire experiment was replicated five times. The results are summarized in table 3.

Table 3.—The aggregation of *Conophthorus coniperda* on models.

	Index mean	Error
40 insects	0.387	0.122
20 insects, 20 models	0.368	0.105
80 insects	0.270	0.016
40 insects, 40 models	0.288	0.103

These experiments suggest that the observed effect of density is a simple one, uncomplicated by previously unsuspected factors of mutual attraction, facilitation, or other phenomena. There is no detectable difference between the aggregation of groups of insects with that of groups composed half of insects and half of dummies. This finding also suggests that the movement of only half the individuals of a group is sufficient to insure that aggregation is completed within the standard running time of 30 minutes.

The Effect of Sexual Differences

When the insects are seen aggregating first in pairs and later in larger groups, the first thought which comes to mind is that the aggregation is of sexual origin. The distinction of the sexes in the living insects is difficult, and indeed was not possible during the first part of these studies. A first testing of the possibility that the aggregation was sexual was done by placing a large number of fresh beetles in a container. Pairs were picked out as they were seen to form. The approaching animal was distinguished from the animal approached. These insects were dissected to establish their sex. The result of these observations is given in table 4.

The observation was repeated later in the course of the studies when it became possible to identify the sex of living beetles (Herdy 1959). The results from tethering beetles of known sex and examining the beetles approaching the captives are given in table 4. There is no indication in these results that a sexual basis of aggregation exists. In spite of the apparent lack of sexual attraction in the development of aggregation, the possibility remained that a sexual difference in overall behavior would be apparent in aggregation.

Table 4.—The sex of aggregating pairs of *Conophthorus coniperda*.

Males approached by males	8
Males approached by females	8
Females approached by males	8
Females approached by females	10
Tethered males approached by males	14
Tethered males approached by females	12
Tethered females approached by males	10
Tethered females approached by females	11

In the previously reported experiments on the effects of density, the use of dummies in equal number with insects was found to give results which compared with those using double the number of insects. A possible way to isolate sexual differences in aggregation at any given density would thus be the substitution of dummies for the beetles of one sex. This experiment was done using 25 males, 25 females and 25 unsexed beetles, each with an equal number of dummies. The experiment was replicated twice, and the results are given in table 5.

It is apparent that the males aggregate in similar manner and degree to the groups of mixed insects comprised of insects taken unsorted from stock. The females aggregate to a lesser extent. Since equal numbers of males and females were involved in aggregation in the free association reported in the previous experiment, it must be concluded that males move more rapidly or are otherwise different from the females in their aggregation. Since this conclusion is different from that suggested by the first observation on this topic, a further observation was made in which the rather suspect dummies were not used.

Groups of fifty—males, females and unsorted insects—were run for 30 minutes in the standard manner. The observation was replicated five times. Far more variation in the results was found than would be expected from the previous observations.

Table 5.—Aggregation of males vs. females of *Conophthorus coniperda* to equal numbers of dummies.

	R	S_{re}
25 males, 25 dummies		
Run 1	0.367	0.045
Run 2	0.286	0.030
25 females, 25 dummies		
Run 1	0.485	0.027
Run 2	0.473	0.031
25 unsorted, 25 dummies		
Run 1	0.395	0.028
Run 2	0.293	0.029

However, the males aggregated to an extent which yielded a mean index of 0.346 with an error of 0.078. The mixed groups showed about the same amount of aggregation with a mean index of 0.365 and an error of 0.083. The females, on the other hand, showed a mean index of 0.483 with an error of 0.039.

The conclusion to be drawn from this further experiment is that males and females do in fact aggregate to a different extent, with the behavior of groups being very close to that of males alone. Interestingly enough, there are indications from the field behavior of the insect that the degree of thigmotropism is greater in the females during or following dispersal. The males and females appear at about the same time, but the females are almost always first to mine the cones (Godwin 1959). Certainly the sex difference in aggregation is not well understood.

An attempt to investigate the effect of individual females in groups of males gave inconclusive results. There is no indication of differing walking speeds or reaction distance between the sexes. There are no apparent chemical factors involved; neither sex has been shown to react to the scent of the other in olfactometer studies.

The Effect of Temperature and Humidity

Because of the inevitable physical relationship between air temperature and relative humidity, it is convenient to investigate the response of animals to the two factors concurrently. It is difficult to manipulate temperature independently of humidity but rather easy to control humidity at any given temperature. As a preliminary to these investigations, replicated runs of 200 insects were exposed for 30 minutes at room humidity of 40% R. H. and in saturated air. The results are summarized in table 6.

The error in this experiment was exceedingly high, but it was evident that humidity did not exercise a marked effect at room temperature.

Using runs of 100 insects, a series of tests of the effect of 40% and 100% relative humidity was run at a series of temperatures from 25° C. to 37° C. Five temperature levels at two relative humidities were tested. A summary of the analysis of the means is presented in table 7. As replications were not significant, means for replications are pooled and their variance assigned to the error term. Interactions are, of course, not separated in this analysis.

Table 6.—The effects of humidity at room temperature on aggregation in *Conophthorus coniperda*[a].

	R	s_{re}
40% R. H.		
Run 1	0.345	0.094
Run 2	0.284	0.166
100% R. H.		
Run 1	0.290	0.144
Run 2	0.249	0.183

[a] 200 insects per determination.

It may be concluded that high humidity promotes aggregation. This finding suggests that the positive thigmotropic response is not connected with the stress of water loss. On intuitive grounds, such a connection might be suspected since it is known that a number of forms take steps which reduce their exposed area under the stress of water loss (Edney 1957). In order to test this idea further, a series of groups of insects was run for 30 minutes, 50 insects to a test at 40% and 100% R. H. in light and dark and with and without cover provided. The insects reacted to cover as before (see section on "limit of aggregation") and the insects at saturation showed no reduction of their reaction. I should conclude that this finding simply confirms the implication of table 7 and suggests that the thigmotropic response is not a function of water balance.

An earlier investigation (Henson 1960) showed that when insects are exposed in a temperature gradient, aggregation tends to take place more frequently in the hotter parts of the gradient. This is the result of insects moving to a warm part of the gradient after being introduced. Only later do the insects move toward cooler zones where they settle in a stable pattern. If insects collide they will form aggregations. Since, immediately after being placed in the gradient, the insects go to a hot area where their walking speeds are high and the probability of collision high, aggregation is more probable while the insects are in the hot part of the gradient. As a result, if one examines a stable distribution of insects in a gradient, those in the warm zone are for the most part aggregated while those in the cooler parts are single.

This finding apparently is inconsistent with the observation that temperature is not effective (table 7). The conditions of the test appear to be at the root of the difficulty. The tests analyzed in table 7 were done with 100 insects per run. The test illustrated in figure 1 suggests that this density is almost great enough to give maximum aggregation at room temperature. A short experiment was done with the insects run at densities of 50 per test at temperatures of 15°, 25°, and 35° C. The experiment was replicated. Under these conditions, temperature was effective. The index of aggregation at 15° C. was 0.620 (s_{re} 0.105). The index at 25° C. was 0.447 (s_{re} 0.056) and that at 35° C. was 0.401 (s_{re} 0.042). The levels reached at 25° and 35° C. are close to the

Table 7.—The effects of humidity at graded temperatures between 25° and 37.5° C. on the aggregation of *Conophthorus coniperda*: Summary of the analysis of variance.

Source	Degrees of freedom	Mean squares	F. ratio
Total	9		
Temperature	4	0.0048	0.70
Humidity	1	0.0649	9.27[a]
Error	4	0.0070	

[a] Significant at 5% level.

limit suggested in figure 1. It might be possible to arrive at test conditions where a graded response at each temperature level could be demonstrated. I feel that this could be done only by selecting density and run time with the greatest care. The point is not, however, critical. The demonstrated effect of temperature supports the conclusions of the earlier study, and that study is felt to be a more adequate outline of the influence of temperature on aggregation. In the earlier paper, humidity was disregarded because of the lack of effect at room temperature. Since the effect of low humidity demonstrated at higher temperatures would tend against the effect of temperature in promoting aggregation, my position would be that both effects are substantial and that it has not been possible to demonstrate the nature of the antagonistic interaction. With adiabatic concurrent change in both factors, the temperature response takes precedence at least over short periods such as those used in the test. This is the only judgment consistent with the behavior of insects in the gradient.

Seasonal Changes

The experiments reported above were all carried out with beetles taken directly from overwintering cones, collected in winter and stored cold until needed. These insects had not fed during their adult lives (Henson 1960), their gonads were inactive, and their flight muscles in normal condition.

The emergence of field populations from the overwintering cones takes place in late April and May. The insects feed on terminal tissue: cones and twigs. They lay their eggs after this initial feeding. During sexual activity, the flight muscles degenerate and the gonads increase in size. It was interesting to determine whether the thigmotropic response was modified during their profound changes in the physiology and behavior of the beetles.

Cones were exposed in cages with a layer of earth to simulate the natural forest floor. The cages were placed outside and moistened from time to time. Emergence of the beetles took place at the same time as field emergence.

The spring-emerged beetles were run in the standard manner. Their thigmotropism was much reduced, but it was not possible to make a standard measurement because of the tendency of the beetles to fly. A photograph of the beetles in a petri plate did not provide a basis for nearest-neighbor statistics because flying beetles did not show on the photograph and hence effective density could not be measured. All one can report is that some aggregations did form, so the reaction was not absent. But the amount of aggregation was very much reduced.

Field-collected beetles feeding within cones prior to the start of egg laying were tested in the standard manner. Replication of this and subsequent measurements could not be provided because of the difficulty of obtaining sufficient beetles in the proper physiological condition. However, from the testing of one run of 30 beetles, the thigmotropic aggregation may be said to be fully reestablished following entry into the feeding cones. The beetles were killed after testing, and it was determined that their flight muscles were not yet degenerated and their gonads not fully developed.

Field collected beetles engaged in mining brood cones were tested. One run of fifteen beetles was all that could be obtained. Thigmotropic aggregation was present. Their flight muscles were degenerated and their gonads active or, in the case of one female, empty.

These limited findings suggest that the only period when the thigmotropic response is greatly reduced is during the emergence from the overwintering cones. At this period the insects are very active and in the field they fly to the tops of the trees. It is possible that there is a corresponding loss of thigmotropism during the move from the feeding cones to the cones where broods are established, but this was not detected in the present limited study.

Further work on thigmotropism in *Conophthorus* might well be directed to the examination of those factors which are involved in the changes of the response with season. In the present study, no manipulation which suspended the reaction was discovered. Feeding the insects on clipped twigs and cones, extended exposure to drying, free water, high and low temperatures, continued light and continuous darkness, starvation and a variety of antennal amputations, blinding, and other surgical treatments failed to suppress the reaction. Each of the treatments modified the degree of aggregation measured at standard room conditions but not to a greater extent than could be explained by the general embarrassment of the treated insects.

DISCUSSION

The sensory basis of the thigmotropism evidenced by the aggregation of *Conophthorus* is obscure. Those physical factors which influence the extent of the reaction seem to do so through their effect on the overall activity of the animal. Light is the only stimulus in which this general observation is open to doubt. Even here, the range over which visual stimuli may possibly be effective is very short. Further testing of the validity of the observation of visual aggregation would be provided if it were possible to increase the range of visual response by the substitution of larger dummies. At this time, it would be conservative to conclude that the observed effect of light on the degree of aggregation is a phototaxis. Presumably the light increases the overall activity of the animals and hence the probability of collision.

The one influence which seems critical in the determination of aggregation is related to the seasonal history of the animals. The sensory mechanism involved in the thigmotropism is probably to be determined only by experiments on insects through the period between the dispersive activity and the

entering of the new cones. It is only during these few days of dispersal that the thigmotropism is lost.

The ecological importance of a reaction which holds a cone-inhabiting insect in a position of maximum physical contact with its surroundings is obvious. As previously pointed out (Henson 1960) the reaction would prevent high temperatures and perhaps other unfavorable influences from driving the insects from their cones.

The laboratory studies outlined in this report have revealed an interesting variability in the thigmotropism of *Conophthorus* which opens a new avenue for determining the mechanism behind its appearance and disappearance during different seasons and different kinds of natural activity. At the same time, the studies offer an interesting model of an aggregating organism which can conveniently be investigated from the statistical or behavioral viewpoints in the laboratory. The aggregation there is only an artifact, but this makes it a particularly convenient behavioral tool since it appears only when the insects are in an open arena where they can be manipulated and observed.

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SUMMARY

1. *Conophthorus coniperda* forms aggregations when confined in an open arena. These aggregations are stable and immobile.

2. The aggregations are not the result of chemical attraction. The aggregative response can be evoked by a variety of dummy objects. The response appears to be largely a thigmotropism.

3. Aggregation is complete within 30 minutes in an open space. About 80 to 85 percent of the experimental populations become involved and the percentage which does not actively do so seems moribund.

4. At low densities, the extent of aggregation is a function of density. This apparently results from the relationship between density and the probability of collision between moving beetles.

5. High humidity promotes aggregation. At low densities, high temperature also promotes aggregation, and lighting the arena results in more aggregation than total darkness. These effects apparently stem from the increase in overall activity which results from the stimuli tested. Such an increase raises the probability of collision between insects.

6. It has not been possible to trigger fixation of individuals by providing effective body contact by an artificial crowding of the insects or by any of a variety of physical objects touched to the body surface. This suggests that either the areas of physical contact which are effective are very localized or some very critical loading of the animal in contact is required.

7. The thigmotropic response is present throughout adult life with the exception of the short dispersive period. Further work on the response could well be concentrated on the conditions under which it is lost and regained in the space of a few days. This should yield useful information on *Conophthorus* itself, as well as providing unusual information on details of thigmotropism as such.

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Relationship of the Crop Capacity to the Depletion of the Fat Body and Egg Development in the Corn Earworm, *Heliothis zea*, and the Fall Armyworm, *Laphygma frugiperda* (Lepidoptera: Noctuidae)[1]

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ABSTRACT

Feeding experiments with the corn earworm demonstrated no correlation between the length of life and the amount of feeding or pupal weight. It is shown that the crop expands in direct proportion with the depletion of fat and the reproductive condition of the imago, the number of matured eggs being particularly important. Egg development and the position of the abdominal organs in relation to the crop are described.

As pointed out elsewhere (Callahan 1958b), there is a regular sequence of continuing change occurring in the gross composition of the reproductive system and fat body of Lepidoptera throughout adult life. Observations on thousands of dissected moths of the family Noctuidae had led to the conclusion that there was a definite change also in the crop size of different specimens. Whether this change was dependent upon the quantity of food taken in, or on some other factor such as the amount of fat or reproductive state of the ovaries in the abdominal cavity, was not known. It was felt that feeding would affect crop size and perhaps also the length of life of the imago and that moths from heavier pupae with more fat might outlive those from lighter pupae providing the moths were held inactive and their feeding controlled.

Comparison of Weight of Pupae and Amounts of Food Accepted with Length of Life of the Corn Ear worm (Heliothis zea (Boddie)) *Imago.*—In regard to feeding, Ditman and Cory (1933) stated that there were no apparent differences in length of life between moths in cages and moths held stationary in holders when the moths were properly nourished. They thus implied that activity has no effect on length of life. Callahan (1958a) believed that activity had an effect and showed that mated, well-fed, caged imagos lived longer (females 8.3 days and males 8.4 days) than unmated well-fed, caged imagos (females 5.8 days and males 5.1 days) but that inactive, unmated, well-fed ones in holders lived almost twice as long (females 16.8 days and males 13.5). The longer life of the active mated moths was explained by the fact that mated and ovipositing caged females fed considerably more often before and during oviposition and spent more time feeding than unmated active ones. The average length of feeding of three mated specimens 5 days old and three mated specimens 10 days old was timed. The 5-day, gravid, mated moths averaged eight feedings in 1 day at 20 seconds per feeding. The 10-day-old moths averaged six feedings at 2.5 minutes per feeding.

Quaintance and Brues (1905) pointed out that bollworm longevity and reproduction were influenced by food available to them. Ditman and Cory (1936) state that the corn earworm, while ovipositing on corn plants, was constantly extending the proboscis and feeding on dew and possibly on some secretion of silks. They also showed that imagos fed solutions of sucrose and glucose lived longer than imagos given only water and that imagos from heavy pupae lived longer than those from light pupae.

In order to see if the pupal weight and amounts of food accepted would affect the length of life, records were taken on the weight of the pupae and the percentage of feeding of the imagos after they emerged. A freshly prepared 10% honey solution was offered to the imagos in holders twice a day, morning and night, until death, as described by Callahan (1958a). A fine sable-hair brush was dipped in the food and each imago allowed to feed until it ceased. The same procedure was followed using distilled water. The tarsi and antennae were always touched with the brush to assure that the food and water were in contact with the chemoreceptors. The

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feeding was calculated by the number of times, at two times a day, the imago accepted food or water. It was believed that if all of these imagos, regardless of weight, were allowed to accept or reject the food from a fixed number of feedings, perhaps some correlation could be demonstrated between the pupal weight, length of life and the amount of feeding. All of the imagos died of old age, and a dissection of each one showed that the fat bodies had been depleted. The results are given in table 1.

These data show that imagos fed a 10% honey solution lived an average of 19.0 days while those given only distilled water lived an average of 11.2 days. This is in keeping with the findings of Ditman and Cory (1936) and with Callahan (1958a). These data further show that the imagos fed honey accepted the food an average of 60.5% of the time while those given water to drink accepted only 49.9% of the time. There was no correlation between the length of life, amount of food or water taken, or the weight of the pupae. In many cases the ones that accepted more food died sooner than the ones accepting less, and in some cases the moths from the heaviest pupae died first. The percentage of acceptance among those given honey varied from 44.1% to 69.2% while the percentage of those given water ranged from 20% to 68.7%. Three facts stood out from this experiment: first, that moths never accepted food on the first day of emergence; second, that as the imagos became older, they accepted the food more quickly and were less likely to miss any of the daily feeding periods; third, as time progressed they continually attempted to drink after the food was consumed; and the response lasted for longer periods of time. Activity or mating was not considered to be a factor in this experiment as all the moths were held stationary in the holders and were not mated.

Table 1.—Comparisons of weight of pupa and percentage of food and water accepted with the length of life of the earworm.

Female (honey)			Female (water)		
Length of life	Weight of pupa in mg.	Percent feeding	Length of life	Weight of pupa in mg.	Percent feeding
13	372.6	69.2	8	264.7	68.7
13	145.9	69.2	9	382.1	50.0
16	386.2	50.0	10	358.1	60.0
17	401.9	44.1	12	352.8	54.1
22	312.7	61.3	15	448.2	53.3
24	398.1	62.5	15	488.1	46.6
24	314.6	62.5	15	301.0	43.3
24	396.6	56.2			
24	326.5	50.0			
25	416.2	62.0			
Male (honey)			Male (water)		
11	451.9	68.2	5	396.6	20.0
13	436.5	65.4	10	402.2	45.0
14	390.0	67.8	11	368.2	35.4
21	370.2	64.3	12	406.5	37.5
25	352.2	56.0	13	448.1	50.0
Average percent of feeding		60.6			46.9
Average length of life		19.0			11.2

Relationship Between the Ovary and Fat Body of the Female and the Crop.—From the above experiment it was concluded that since normally feeding moths, whether young or old, did not overly expand in body size there was possible a volumetric change in the crop not dependent upon the volume of liquid the moth imbibed but conversely dependent upon the allowable area for expansion of the crop within the abdomen as determined by the fat body and gravid condition of moths of different ages.

In order to test the above hypothesis a study was made of the ovary of the female and observations were made on the development of eggs and their relationship to the internal abdominal space.

Figure 1 is a diagrammatic representation of egg development within the ovarioles based on counts in 50 female earworms. Egg development is quite consistent within the earworm and each day represents what would normally be the egg development for a moth of the approximate age at 80° F. Eggs develop at the same rate in each of the eight ovarioles.

One-day-old nonmated moths do not have eggs with chorion but only those intermediate eggs without the chorion. These eggs have nurse cells that have become very minute or have disappeared (fig. 1, 1-day, black eggs.) These intermediate eggs have been referred to as unridged (Callahan 1958b) and they vary from 10 to 15 per ovariole in newly emerged moths.

Eggs of 2-day-old moths begin to show the development of chorion with ridges (fig. 1, lined eggs). These mature eggs at this stage range from 10 to 20 per ovariole, while unridged eggs vary between 25 and 30. At this stage no mature eggs have progressed posteriorly past the calyx of the oviduct into the single oviductus communis.

The 3-day-old moth, which represents a type de-

scribed later for category 5, may have from 60 to 80 ridged mature eggs, with the unridged eggs averaging about 40 per ovariole. It should be noted that in such gravid unmated females the eggs not only fill the calyx and oviductus communis but also mature in an anterior direction past the end of the second loop of the ovariole. This is not usually the case, for in most moths, nonmated (4 days) or mated (5, 6, 7, and 8 days) the mature ridged eggs do not ordinarily occur past the end of the second loop. The end of the second loop is considered by the author to be the posterior end of the pedicel. There is no constriction of the ovariole wall between the matured eggs in the pedicel area. The characteristic constriction of the wall which occurs between immature eggs, disappears as more and more eggs mature and fill the ovariole in an anterior direction. The unridged eggs in this ovariole are small and it is possible that this type of unmated moth is maturing eggs with chorion at this earlier age faster than they are absorbing unridged eggs without chorion as described by Callahan (1958b).

In mated moths from 5 to 8 days old, the oviductus communis, calyx, and the ovariole up to the end of

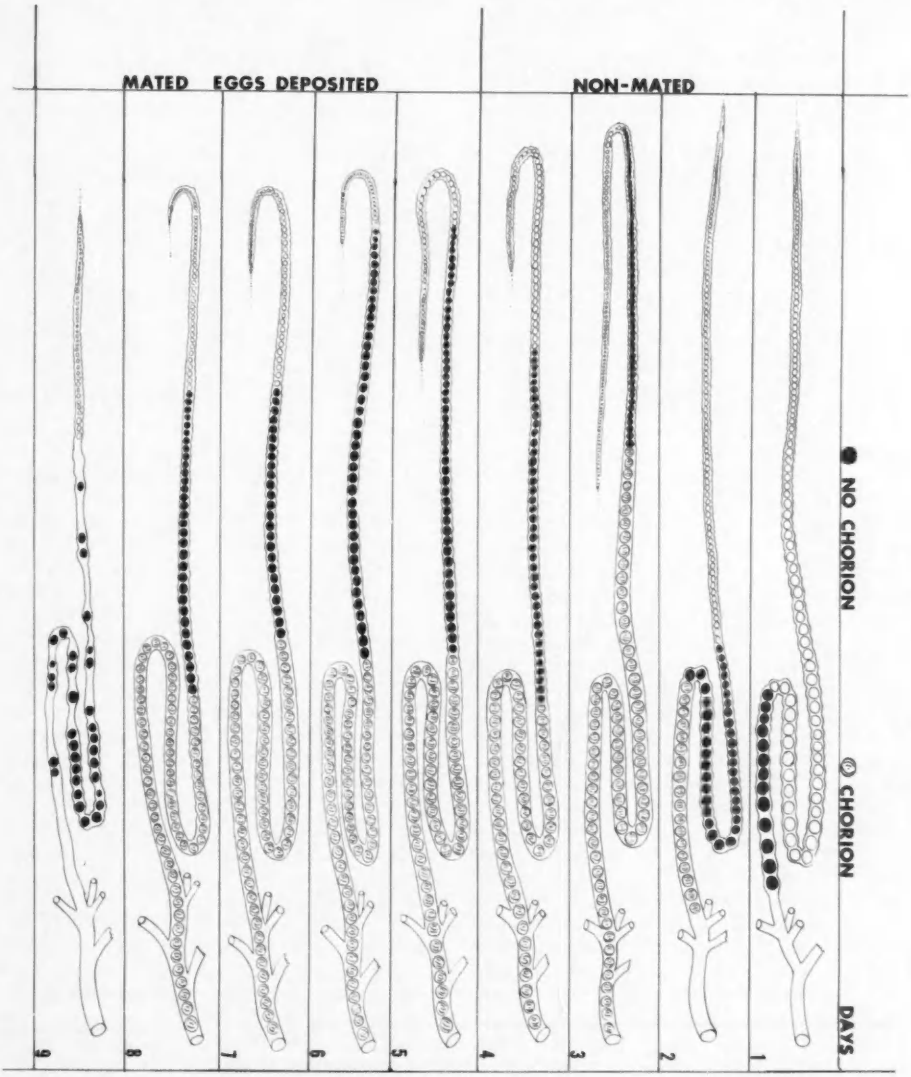


FIG. 1.—Typical ovarioles (diagrammatic) of corn earworm specimens reared at 80° F. and from 1 to 9 days old. Lined eggs represent mature eggs with chorion, black eggs intermediate eggs without chorion, and unmarked eggs the developing eggs with nurse cells in the germarium and vitellarium.

the second loop are filled with mature eggs, the range being from 30 to 60 eggs per ovariole. It should be understood that the moths were killed in the evening, and that had they been killed in the morning after oviposition the mature egg count would have been lower. Fifteen mated moths dissected after a night of oviposition in a behavior cage invariably yielded less than 20 eggs per ovariole.

Callahan (1958b) showed that in mated moths the number of eggs both with and without chorion remained fairly constant until the end of life when most of the eggs had been deposited, whereas nonmated moths that did not oviposit absorbed their eggs without chorion. A certain number of eggs with chorion in nonmated moths are squeezed out at old age or just before death. Such an occurrence is not considered oviposition by the author, but is probably due to a buildup of pressure in the ovarioles since the mature eggs are not absorbed. This is consistent with the observations of Cook (1930) on another noctuid, *Agrotis orthogonia* Morr.

Mated moths that have deposited all of their mature eggs (9 days) show the ovarioles to be in a collapsed condition and contain very few unridged eggs. Those that are present are usually misshapen. In such moths the ovaries take up very little of the body cavity.

The earworm does not mate on the night of emergence nor the first night following emergence (Callahan 1958a). The total egg deposition for 55 pairs of earworms from the 3d day when deposition began until the 15th day, when the last female died, was totaled and plotted (fig. 2). The results show that by far the greatest number of eggs was deposited from the 4th to the 8th day. This is consistent with the condition of the ovaries of dissected mated moths from 4 to 8 days old. All of this work was carried out in air conditioned rooms at 80° F. It is known that at lower temperatures the imago's life is prolonged and egg development slowed down. Jacobson and Blakely (1959) have shown this to be the case for the army cutworm, *Chorizagrotis auxiliaris* (Grote).

Hemisections were made and observations taken on female earworm specimens of various ages and reproductive states. The moths were prepared by killing with ethyl acetate and fixing in 10% formalin. The specimens were then easily bisected down the medial line with a razor blade. All organs remained in place and there was no derangement of any organ due to the pressure from the cutting blade.

Five moths 1 day old, five 2 to 4 days old and five 5 to 8 days old that were mated were killed and examined. The remaining five, three of which were mated, were allowed to die of old age before fixing. All of these moths had been allowed to feed naturally in a behavior cage. The female moths could be divided into 5 distinct categories:

1. Hemisections of 1-day-old female moths showed the abdomen to be so packed with fat that the

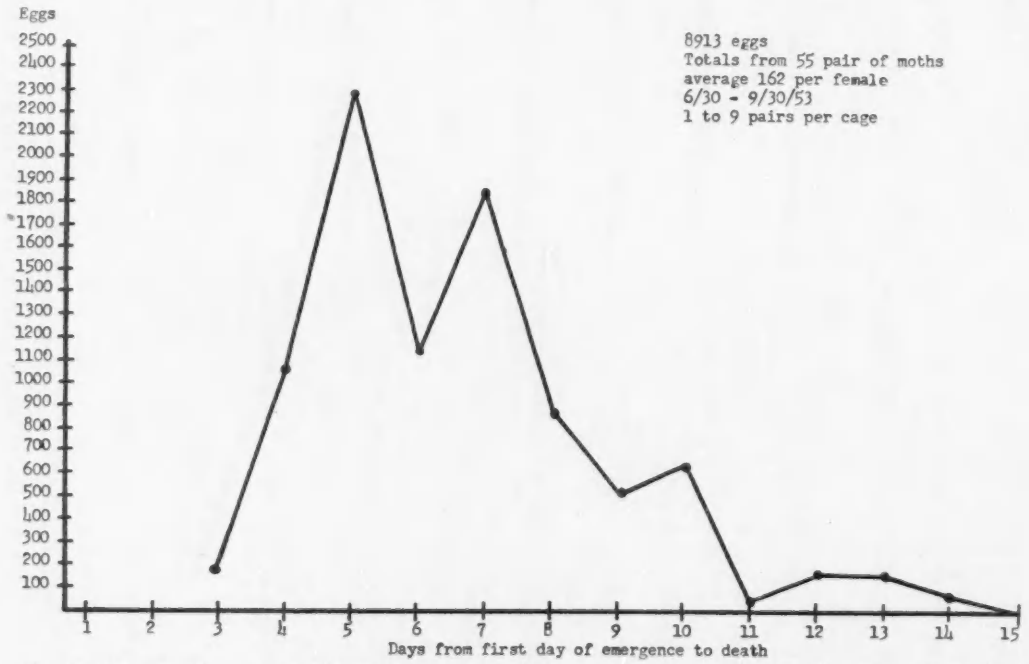


FIG. 2.—Successive days upon which 8,913 eggs were laid by a population of 55 pairs of earworm moths from the time of emergence until death. Each point represents the total of all eggs laid by the caged imagos on that day of their life.

crop was folded as a flat membranous sheet and extended from the area where the esophagus enters the abdominal cavity below the tympanum of the metathorax and up along the inside of the dorsal abdominal wall for 3½ segments. In these moths there is no crop expansion measurable and the crop can only be located with great difficulty. No mature eggs are present.

2. Hemisections of 2- to 4-day-old unmated moths showed a hollow sector, distinctly outlined by the sheet of the crop wall, at the anterior dorsal portion of the abdominal cavity. The expanded crop in all such fixed specimens followed exactly the contour of the surrounding fat body and other organs such as the cervix bursae and ventriculus. Measurements taken of the crop, from the anterior wall to the posterior wall, averaged just a little over 0.8 mm., the range being 0.7 to 1.3 mm.

3. Hemisections of mated, 5- to 8-day-old moths showed a variable crop diameter dependent upon the condition of the ovary. In recently mated 5-day-old moths (fig. 3), which had not deposited eggs, the whole abdomen was filled with mature eggs to such an extent that the abdominal wall was expanded by 1.8 mm. in circumference. It was very difficult to locate the flat sheet of crop in such a moth. At the other extreme were moths 8 days old which had oviposited many of their mature eggs and in which the fat body was almost depleted. This type of moth could be immediately identified for it had a very stable crop diameter of 2 mm.

4. Hemisections of mated moths aged 9 days or older (fig. 4) showed the abdominal cavity to be depleted of the fat body and mature eggs and to have a crop diameter of 3 mm. In this type of specimen, the crop is almost completely expanded and fills much of the dorsal anterior portion of the cavity. As in the other categories with expanded crops, the crop wall exactly followed the contours of the surrounding fat body and other organs.

5. A fifth type of moth was noted and similar moths have shown up occasionally among the thousands of earworms dissected by the author. This type was unmated but had an unusually large number of mature eggs. The ovariole of this type is represented by the 3-day-old ovariole, nonmated moth (fig. 1). As in category 1, the crop is sheetlike and extremely hard to locate.

Relationship Between the Crop and Other Organs of the Female.—It is readily seen from the foregoing that the area of the abdominal cavity occupied by the crop is dependent upon the fat body and the condition of the ovaries of the female. A third factor, not so important in the earworm, is the space occupied by the spermatophore in mated females. It was noted that in gravid females the bursa copulatrix and contained spermatophore (fig. 3 B and C) are held against the dorsal wall of the abdominal cavity by the mature eggs (fig. 3 A). In old females with depleted fat and collapsed ovarioles the bursa and contained spermatophore (fig. 4 H and I) drop down and lie ventrally on the ventriculus (fig. 4 G). The collum of the spermatophore does not expand the cervix bursae and is very thin; however, at one period after copulation, while the spermatophore corpus is expanded with sperm, considerable abdominal space is filled with the rounded corpus bursae (see Callahan 1958b). This period lasts for about 20 minutes (Callahan, unpublished data). The earworms do not feed during this time so it is doubtful if it is important in relation to crop volume since the spermatophore corpus soon collapses and occupies very little area.

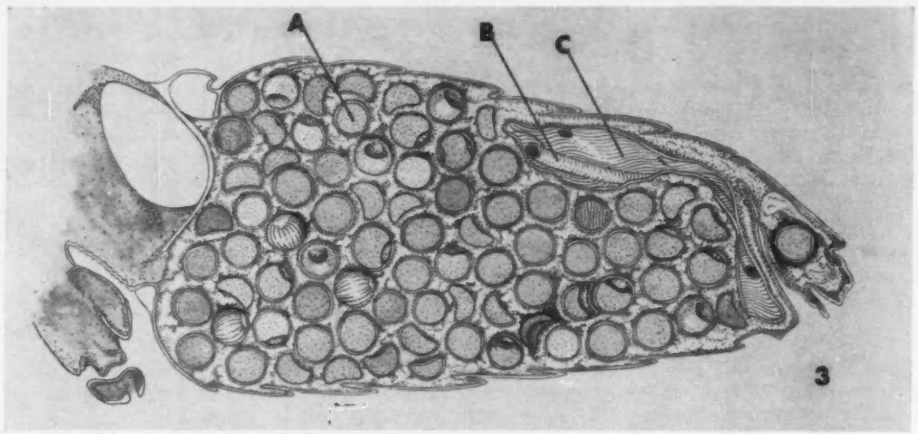
In moths 9 days of age or older (type 4) with a 3-mm. crop diameter the rectum also becomes very prominent and expanded (fig. 4 E) and fills the space along the dorsal wall which earlier is filled by the cervix bursae in young gravid females. In younger females the rectum, like the crop, is difficult to locate as it is flat and concealed among the fat body and eggs.

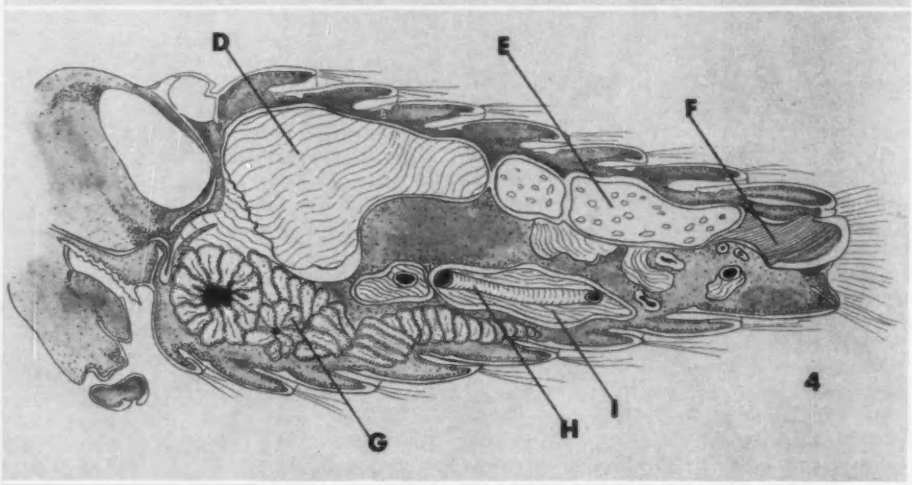
Relationship Between the Crop and Organs of the Male.—The continuing change in volume of the crop in the male is not so difficult to detect as in the female for it is not obscured by changes of egg development but is dependent only upon the depletion of fat.

The crop presents the same picture in 1-day-old males as in the female. The abdominal cavity is packed with fat and the crop is folded as a flat, membranous sheet extending from the esophogeal area along the dorsal wall for 3½ segments. As in the female, there is no crop expansion. Hemisections of 5-day-old males with half the fat depleted showed crop measurements ranging from 1.5 mm to 2.5 mm. in diameter, and males that had died of old age had crops that measured from 3 mm. to 3.5 mm. in diameter.

Measurements of the diameter of the crop in males were complicated by the fact that the aedeagus (fig. 5) was retracted into the abdominal cavity much further than might have been expected. In all specimens dissected the anterior end of the aedeagus and the protractor muscle (K) is retracted up into the crop area so that the crop (J) actually folds around the aedeagus. Obviously the full crop capacity could be realized only when the aedeagus is protracted, which occurs only during mating when the moth does not feed. The aedeagus is retracted as far forward as the membrane between the second and third abdominal sclerites and even the saccus and vinculum (T), upon which the retractor muscle (L) inserts, retracts forward to the intersegmental membrane between the fifth and sixth abdominal sclerites. The aedeagus of the earworm is extremely long because it has no endophallic caeca (Callahan and Chapin 1960). Measurements were thus taken along the dorsal area of the crop that expands above the aedeagus to the rectum (M). As in the females the rectum is expanded in old moths with depleted fat.

The male reproductive organs, testes (P), primary





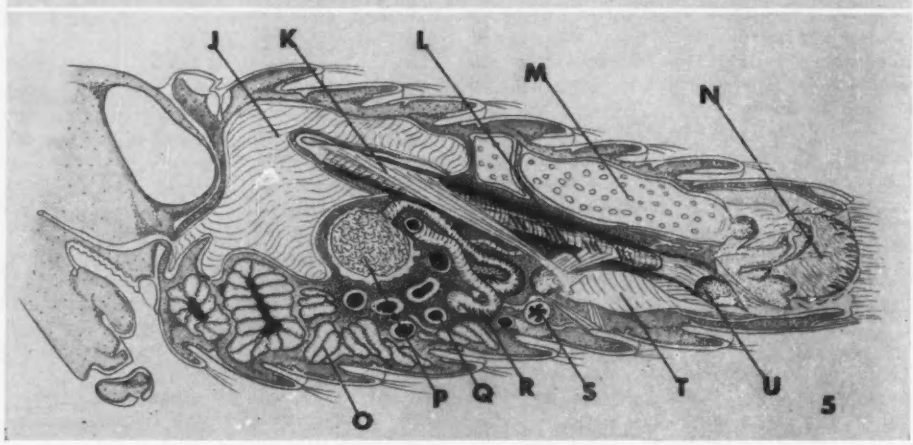


FIG. 3.—Hemisection of gravid 5-day mated female. a. Mature eggs with chorion in ovarioles; b. Spermatophore collum; c. Cervix bursae of bursa copulatrix.

FIG. 4.—Hemisection of 10-day mated female after eggs have been deposited. The fat body and ovaries fill spaces between other organs but are omitted to show the organs more clearly. d. Crop; e. Rectum; f. Ovipositor; g. Ventriculus; h. Spermatophore collum; i. Cervix bursae of bursa copulatrix.

FIG. 5.—Hemisection of 8-day male, fat body omitted. j. Crop; k. Protractor muscle of aedeagus l. Retractor muscle of aedeagus; m. Rectum; n. Clasper; o. Ventriculus; p. Testes; q. Ductus ejaculatorius simplex, primary segment; r. Ductus ejaculatorius duplex; s. First secretory area of the primary segment of simplex; t. Vinculum; u. Posterior end of aedeagus.

segment of the simplex (Q), duplex (R) and the first secretory area of the primary simplex (S) are all coiled up between the aedeagus and ventriculus (O). The testes and ducts take up considerable space and do not allow the ventral portion of the crop to expand more than 2.3 mm.

Crop Measurements in the Fall Armyworm (Laphygma frugipherda (J. E. Smith)).—Since the measurements of the earworm were taken on reared caged specimens and could not represent what occurs in feral populations, it was felt that crop expansion should be checked in a light-trapped population. No earworms were available, so measurements were taken on fall armyworms to see if crop expansion followed a similar pattern in a noctuid with comparable length of life and feeding habits. The fall armyworm moths were taken from a 700-specimen sample caught in August 1960 and fixed in 10% formalin. Fifty females and 25 males were checked and measurements in millimeters taken to the first decimal place. A larger female sample was used because it was necessary to break it down into groups according to the reproductive state. The moths were graded for fat body depletion from 1 to 4, with grade 1 representing young moths and grade 4 those with all fat depleted. The measurements for crop diameter are given in table 2.

The averages show clearly that as the moths age the crop diameter expands directly as the fat body is depleted, ranging from 1.18 in young (grade 1) moths to 3.15 mm. in old (grade 4) moths. Surprisingly, almost every possible reproductive condition was found in a sample of 50 female fall armyworms picked at random out of 700 specimens. These ranged from one old, mated specimen (grade 4) that apparently had not oviposited before capture, as the ovarioles were packed with eggs, to a specimen (grade 2) in which the corpus of the spermatophore was still expanded with sperm, and two specimens that were copulating when caught.

The spermatophore in the fall armyworm is more important in reducing crop volume than in the corn earworm. The corpus bursae is heavy and saclike After mating it contains not only the spermatophore corpus but also three-fourths of the collum, which bends springlike against the walls of the bursa and keeps it expanded. This condition exists even after the corpus has collapsed. Note that the crop size (table 2 and footnotes thereto) of two grade-4 moths, mated three times but with no eggs, was smaller (2.2 and 2.5 mm.) than the average (2.85 mm.) for the six moths, the other four of which had mated but once. Mature eggs that have not been deposited from old moths reduce the crop volume (2.0 mm.) considerably. One grade-2 moth with eggs and containing two spermatophores, one of which was still filled with sperm—indicating that it was killed within an hour after mating (Callahan, unpublished data),—had no crop measurement and was comparable to a young, grade-1 moth. The two moths in the process of mating were grade 1, and the previously mated grade-1 moth that contained a spermatophore had no crop volume.

The increase in crop size, from an average of 1.17 in young (grade 1) moths to 3.90 in old (grade 4) moths that had not mated and matured no eggs is quite evident.

The crops of the fall armyworm males were easily measured as the aedeagus is short, with a rounded endophallic caeca that does not retract anteriorly into the crop as in the earworm.

DISCUSSION AND SUMMARY

Ditman and Cory (1936), in testing responses of the earworm to sugars, noted that in any series of moths tested, the greatest proboscis response (not feeding but extension alone) never occurred on the first day and that in some series the response increased gradually to the last day. In most of their work, however, the age of the moth seemed to have

Table 2.—Crop measurements (in millimeters) of the fall armyworm in relation to grade of fat body and reproductive state.

Reproductive condition	Grade 1 fat body			Grade 2 fat body			Grade 3 fat body			Grade 4 fat body		
	No.	Crop diameter		No.	Crop diameter		No.	Crop diameter		No.	Crop diameter	
		Range	Avg.		Range	Avg.		Range	Avg.		Range	Avg.
Males				12	1.2-2.6	1.94	12	2.5-3.1	2.84	1		3.84
Females:												
With no eggs												
Unmated	4	0.0 -1.9	1.17	8	1.8-3.0	2.51	8	2.5-3.1	2.96	3	3.5 -4.2	3.90
Mated	1		1.60	1		2.10[b]	1		1.60	6	2.2[c] 2.5[c] -3.8	2.85
With eggs												
Unmated	2	0.9[a]-1.9	1.40	7	1.1-2.5	1.98						
Mated	4	0.0[a]-1.2	0.55	4	0[b,d]-1.5	1.10				1		2.00[e]
Totals and averages	11		1.18	32		1.93	21		2.47	11		3.15

[a] Individual in process of mating.
[b,c] Females with two or three spermatophores, respectively, in bursa (all other mated females had one spermatophore only).
[d] Corpus of one spermatophore fully expanded and filled with sperm.
[e] Ovarioles completely packed with mature eggs.

little effect on the degree of the response, greatest response occurring on any day after the first. All of this work was carried out with moths placed in holders within 24 hours of emergence and they were thus not mated. These data certainly help to explain their response phenomenon. There is considerable doubt that 1-day-old moths, with no crop valume, would have the physiological prerequisite for a feeding response, if such a prerequisite were dependent on crop size. On the other hand, there was a gradual increase in the response of some series to the last day. Such series probably represented those moths in which the fat was gradually being depleted, perhaps males or nonmated females with no mature eggs, thus the crop expanding with age could initiate greater and greater feeding responses. In cases where age had little effect on response the probability was that those individuals were of considerable variability in the number of eggs developed and the response was proportionally variable. Certainly, as has been shown, a moth with many mature eggs might be old but have quite a small crop volume to fill.

Callahan (1958a) has pointed out that ovipositing earworms visit the feeders much more often than nonovipositing moths. This would be logical in view of the smaller crop area of gravid females. Such moths never stay long at the feeder but return again and again. Ditman and Cory (1933) further point out that occasionally moths will never respond even to extremely high concentrations of most attractive sugars. These variabilities may well be due to the crop volume at any particular age or reproductive state.

In regard to the physiology of feeding, a disease entitled by this author the "black side disease," is of considerable interest. Ditman and Cory (1933) commented on a peculiar condition of the abdomen characterized by a hardening and dark color. This occurred among a few of their moths feeding in holders and the moths died in a short time. It seems to be a condition more associated with inactive moths in holders rather than active caged moths. Fifteen specimens died of a similar malfunction during these feeding experiments whereas only two have been discovered among hundreds observed in cages by the author.

Investigation of the peculiar "black side disease" revealed several interesting facts. The characteristic symptoms of the disease are a gradual darkening of the sclerites and dropping of scales from the sides of the moths between the fourth and sixth abdominal sclerites, but never dorsally or ventrally. As the disease progresses, usually over a 2-day period, the sides finally become extremely hard and very black. Internal dissections reveal the ventriculus at the posterior end to be affected and apparently blocked by a hardened and constricted condition. Moths at the final stage of the disease become extremely gluttonous and will feed without cessation. Due to the blocking of the ventriculus moths that are allowed to continue feeding will swell and swell until the tissues and crop tear, and honey solution leaks out between the sclerites. The inside of the abdominal cavity becomes saturated with the solution and a considerable portion of it is also excreted. Shortly after such a feeding the honey solution begins to crystalize and the moth hardens and dies. The blocking of the ventriculus and hardening of the sides of the moth apparently upsets the physiological mechanism for determining feeding capacity and the moth literally drinks itself to death.

In certain insects air sacs have often been presumed to have a secondary function of filling up the extra space in the abdominal cavity as fat is depleted, a requirement for preventing the organs from rattling around, so to speak. Since such statements are theoretical, this may or may not be, but it is interesting to note that in some species of the noctuid subfamily Catocalinae, which are quite attracted to fermenting sugars, the crop is reduced, and much of the body space is filled with air sacs. In the subfamilies to which the earworm and fall armyworm belong there are no such large air sacs. We might presume, then, that the large, folded crop to some extent substitutes for the lack of air sacs and thus serves the additional purpose of filling the gradually expanding spatial areas of the abdominal cavity.

In summary we may conclude that length of life does not correlate with pupal weight or amount of feeding or drinking, that the percentage of food acceptance varies among specimens tested and the imagos given food accept more often than those given water, that as they grow older they accept more quickly and imbibe for a longer period, and that they do not feed immediately after emergence.

It is further shown that the crop expands in direct proportion to the depletion of fat and reproductive state of the imago, the condition of the ovaries and development of mature eggs being particularly important, and that most mature eggs are deposited from the 4th to the 8th day at 80° F. Relationships between the crop and other organs are described and the spermatophore is considered a factor in crop expansion in the fall armyworm. It is suggested that crop expansion is not dependent upon the amount of liquid imbibed but, conversely, the liquid imbibed depends upon allowable crop space as determined by fat and reproductive state of the imago. It is further shown that when a disease, "the black side disease," upsets the cut-off mechanism, the moth literally expands itself with liquid until death results. Lastly, it is pointed out that the expanding crop may also serve to fill the expanded spatial area brought about by fat depletion as do, presumably, the air sacs in the Catocalinae subfamily of the Noctuidae and other insects.

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Rondani's "Dipterologiae Italicae Prodromus" [1]

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ABSTRACT

Volumes VI-VIII of Rondani's important eight-volume study of Diptera are a somewhat confusing series of fascicles published both separately and in journals. Available sets are incomplete or a mixture of the two sources, with consequent confusion in citation. An annotated analysis in parallel columns compares the two sets of references.

The "Dipterologiae Italicae Prodromus" (1856-1880) by Camillo Rondani is one of the most significant and often-cited works on the classification of the Diptera. All parts were published separately, with perhaps one exception, and all but two also appeared in journals. Unfortunately, available sets of the Prodromus are often incomplete or of mixed origin, and consequently misleading. In 1914, the publishing house of W. Junk, then at Berlin, brought out a "Facsimile-Edition" of the Prodromus, in seven volumes (Vols. VII and VIII combined as one). This is probably the only copy of Rondani's classic available in many libraries. However, it too is incomplete, and furthermore it was copied partly from separate works and partly from journals.

The arrangement and pagination of the entire work are outlined below, so that any available part may be correctly identified in the general pattern. Only a few details remain uncertain, and these are so indicated. The contents of the pages are generally identical for the corresponding separate works and journal articles, so that references for one can usually be calculated from the other, if only one is available. Any deviations caused by rearrangement of material are noted. In most cases, it would be well for taxonomists to cite both the separate work and the corresponding journal article, partly for the benefit of the reader who may have only one or the other before him, and partly because it is seldom clear which has priority.

For brevity in discussion, several works that are cited repeatedly are referred to by single words, as follows:

Neave = "Nomenclator Zoologicus," vols. 1-4, 1939-40.

Katalog = "Katalog der Palaärktischen Dipteren," (vol. 3, 1907, by Bezzi and Stein; vol. 4, 1905, Holometopa by Becker).

Atti = Atti della Società Italiána di Science Naturáli (Milano).

Bullettino = Bullettino della Società Entomologica Italiána (Firenze).

The initial volume of the Prodromus divided the Diptera into 32 families and 84 stirpes (= subfamilies), with their included genera. Volume II covered the first three families, with ten stirpes. Volume III began the large fourth family, the Muscidae, with stirps XI and part of XII, and succeeding volumes included the remainder of stirps XII and stirpes XIII through XXV, the last published in 1880 after Rondani's death in 1879.

The first five volumes of the Prodromus (1856-1862) were published only as separate works. Their dates and pagination are unquestioned, and the references are given here only for completeness. The difficulties arise in the interpretation of Volumes VI, VII, and VIII, which appeared in three different journals as well as separately. Citations in the literature appear to have been influenced by what was available to the individual taxonomists, with or without awareness of the existence of other versions.

Two points of terminology have undoubtedly added to the confusion: (1) The term "Pars" was used for successive parts of the series on the "Species Italicae . . .," which began with Volume II of the Prodromus, and was also used as equivalent to "Volume" of the Prodromus, for Volumes VI and VII; (2) the term "Fascicle" was applied both to the four parts of Volume (as "Pars") VII of the Prodromus, and to four papers that appeared from 1865 to 1871 under the title of "Diptera Italica non vel minus cognita . . ." The latter four, which are considered part of Volume VI of the Prodromus, contain material supplementary to earlier volumes.

[1] Accepted for publication March 31, 1961.

Table 1 outlines the various parts of the Prodromus, the existence of separate works or journal articles, and the source from which the Facsimile-Edition was prepared. On succeeding pages are the details regarding the volumes and parts.

Table 1.—Outline of the Dipterologiae Italicae Prodromus in Separate, Journal, and Facsimile Publication.

Prodromus	Separate publication	Journal publication	Source of facsimile-edition (1914)
Vols. I - V	1856-62	None	Separate
Volume VI			
Dipt. Italica, Fasc. I	1865	1865	Lacking
Dipt. Italica, Fasc. II	1865	1865	Lacking
Species Italicae, Pars V (Anthomyinae)	1865 [1866?]	1866	Lacking
Dipt. Italica, Fasc. III	[1868?]	1868	Lacking
Dipt. Italica, Fasc. IV.	[separate?]	1871	Lacking
Species Italicae, V (Anthomyinae, revised)	1877	None	Separate
Volume VII			
Fasc. I, Scatophaginae	1866	1867	Journal
Fasc. II, Sciomyzinae	1868	1868	Lacking
Fasc. III, Ortalidinae, Ortaloidi	1869	1869	Journal
Fasc. IV, Ortalidinae, Tephritoidi	1870-71	1870-71	Journal
Sciomyzinae (revised)	1877	1877	Journal
Volume VIII			
Stirps XXI, Tanipezinae	1874	1874	Journal
Stirps XXII, Loncheinae	1874	1874	Journal
Stirps XXIII, Agromyzinae	1875	1875	Journal
Stirps XXIV, Chylizinae	1876	1876	Journal
Stirps XXV, Copromyzinae	1880	1880	Journal

"Dipterologiae Italicae Prodromus"

1856. Vol. I. "Genera Italica ordinis dipterorum ordinatim disposita et distincta et in familias et stirpes aggregata." 226 pages, plus two unnumbered pages. (No journal article.)

1857. Vol. II. "Species Italicae ordinis dipterorum in genera characteribus definita, ordinatim collectae, methodo analitica distinctae, et novis vel minus cognitis descriptis." "Pars Prima: Oestridae: Syrpfhidae [sic]: Conopidae." 264 pages, 1 plate. (No journal article.)

1859. Vol. III. "Species Italicae . . ." "Pars Secunda: Muscidae, Siphoninae et (partim) Tachininae." 243 pages, plus one page of explanation of figures and one plate. (No journal article.)

1861. Vol. IV. "Species Italicae . . ." "Pars Tertia: Muscidae, Tachininarum Complementum." 174 pages. (No journal article.)

1862. Vol. V. "Species Italicae . . ." "Pars Quarta: Muscidae, Phasiinae—Dexiinae—Muscinae—Stomoxidinae." 239 pages. (No journal article.)

PRODROMUS, VOLUME VI

Separate works

1865 [1866?]. "Species Italicae ordinis dipterorum vel annotata observationibus nonnullis additis." "Fasc. I. Oestridae—Syrphidae—Conopidae." Pp. [1]-20.

1865. "Diptera Italica . . ." "Fasc.. II, Muscidae." Pp. [21]-59. — On p. [60] is brief note that this is an "Estratto" from the Atti, Vol. VIII, and the imprint "Milano, 1865." The note applies to both fascicles, as the pagination is continuous and both are indexed together on pp. [57]-59. I do not know whether the two fascicles were issued separately or only in conjunction with the following part on Anthomyinae (cf. its title page, "cum supplemento"), which constitutes the major part of the Prodromus volume VI.

Journal articles

1865 (May). (Title as in separate work.) Atti Soc. Ital. Sci. Nat. 8: [127]-146.

1865. (Title as in separate work.) Atti Soc. Ital. Sci. Nat. 8: [193]-231.

Both Neave and the Katalog cite the Atti references for these two fascicles. The Facsimile-Edition does not include them.

1865 [1866?]. "Species Italicae ordinis dipterorum a Prof. Camillo Rondani collectae, distinctae, et in ordinem dispositae novis, vel minus cognitis descriptis. Pars Quinta, Anthomyinae." "Dipter: Ital: Prodromi cum supplemento, Pars [= Volume] VI." Title page (unnumbered) and pp. [1]-151. — The title page given here is probably the main title page for Volume VI, including the first two supplements, which are bound with it in the copies that I have seen. Page [1] of the text corresponds to p. 68 of the journal article and carries the same title as the latter (see opposite). The main title page has 1865 as the imprint date, but on p. [152] is the note that this is an "Estratto" from the Atti, Vol. IX, and the imprint "Milano, 1866." Page [1] has the parenthetical notation that the paper was presented at the meeting of Nov. 26, 1865, and this is so late in the year that publication in 1866 is probable.

1866 (June). "Anthomyinae Italicae collectae distinctae et in ordinem dispositae a Prof. Camillo Rondani." Atti Soc. Ital. Sci. Nat. 9: [68]-217. — The slight discrepancy in total pages between journal and separate arises in the position of the Index and does not affect comparison of text. The last page of text is 214 in the journal and 147 in the separate work. In the journal, the Index is on pp. 215-217, whereas in the separate p. [148] is blank and the Index is on pp. 149-151.

Neave and the Katalog (vol. 3, 1907) both cite 1866 and the Atti reference for the new genera involved. This part is not con-

tained in the Facsimile-Edition, in which Volume VI is made up of the 1877 revision of Pars Quinta (see below).

[1868?]. "Diptera Italica . . . Fasc. III." Pp. [61]-94. — This supplement includes species in Oestridae, Syrphidae, Conopidae, and Muscidae.

1868 (June). (Title as in separate work.) Atti Soc. Ital. Sci. Nat. 11: [21]-54.

Both Neave and the Katalog cite the Atti reference for this fascicle. The date of the separate work is unknown to me. Presumably, it did not appear until 1868, but its pagination is continuous with the first two fascicles. In the copy before me, there is no statement that it is an "Estratto" from the Atti, though the pages are of identical composition. Like the first two supplements, it is not included in the Facsimile-Edition.

No separate form of the fourth fascicle has been seen, though it may exist.

1871. "Diptera Italica non vel minus cognita descripta aut annotata." "Fasc. IV. Addenda Anthomyinis Prodr. Vol. VI." Bull. Soc. Ent. Ital. 2: 317-338. — This was published in the fourth trimestre (Oct. to Dec.) of the Bullettino for 1870. The date of publication has usually been cited as 1870, but the imprint date on the cover of that trimestre is actually 1871.

Fascicle IV as such is lacking in the Facsimile-Edition. Its contents were fused with the earlier (1866) publication on the Anthomyinae, plus a few new genera and species, to form the 1877 issue of Volume VI (see next entry). Incidentally, one species from Fascicle IV, *Spilogaster sylvanus* (p. 332), was missed in putting together the 1877 paper.

1877. "Species Italicae ordinis dipterorum ordinatim dispositae, methodo analitica distinctae, et novis vel minus cognitis descriptis." "Pars Quinta. Stirps XVII —Anthomyinae." Parma. Pp. 304, the text beginning on p. [5].

This is a revision and expansion of the first publication on the Anthomyinae (1866). Into it are incorporated the anthomyiids published in Fascicle IV (1871), except for one species as noted above, as well as a few new genera (*Macrorchis, Hylephila, Hammomyia, Orchisia*) and new species. Only these new genera and species are properly cited from the 1877 work. This is Volume VI of the Facsimile-Edition.

Not published in a journal.

PRODROMUS VOLUME VII

1866. "Scatophaginae Italicae collectae distinctae et in ordinem dispositae." "Dipterol. Ital. Prodromi Pars [= Volume] VII, Fasc. I." Pp. [1]-51. — This includes Stirps XVIII. On p. [52] is the statement that it is an "Estratto" from the Atti, Vol. X, and the imprint "Milano, 1866."

1867. (Title as in separate work.) Atti Soc. Ital. Sci. Nat. 10: [85]-135.

This work has sometimes been credited to the Prodromus, Vol. VII (1866) and sometimes to the Atti (1867). The Katalog (vol. 4, 1905) is not consistent, usually citing the Prodromus, but sometimes giving both.

1868. "Sciomizinae [sic] Italicae collectae, distinctae et in ordinem dispositae." "Dipterol. Ital. Prodromi Pars VII, Fasc. II." Pp. [1]-58. — Stirps XIX. On p. [59] is the statement that it is an "Estratto" from the Atti, Vol. XI, and the imprint "Milano, 1868."

1868 (Oct.) "Sciomyzinae Italicae . . ." Atti Soc. Ital. Sci. Nat. 11: [199]-256.

Neave cites the Atti reference, and the Katalog cites the Prodromus. Probably the separate has priority. The difference in spelling of the group name suggests that the separate appeared first, and that the error was then corrected for the journal article.

This sequence is further supported by the fact that the journal article has an inconspicuous, single-line, italicized title, as if hastily inserted, whereas that of the separate is a well-spaced, two-line title in two sizes of bold-faced capital letters. It would certainly have been available for printing before October (date of the journal), because it was presented at the meeting of June 28, 1868. The Facsimile-Edition contains a revised version of this fascicle, published at a later date (1877, q.v.).

1869. "Ortalidinae Italicae collectae, distinctae et in ordinem dispositae." "Dipterologiae Italicae Prodromi Pars VII—Fasc. 3." "Linea A. Ortaloidi." Pp. [1]-37. — Stirps XX. The pagination of text material is the same as in the journal article. The first two parts of the title, which form the upper half of p. [5] in the journal, have been moved in the separate work to pp. [1] and [3], respectively, as title and subtitle. The actual text begins on p. [5] in both. This is not marked as an "Estratto" from the Bullettino, and was cited in the Katalog as a separate publication.

1869. (Title as in separate work). Bull. Soc. Ent. Ital. 1: [5]-37. Published in the first issue, for January, 1869. Osten Sacken (1903, Record of my Life Work in Entomology, p. 151) states that the publication of the first part on the Ortalidae "had taken place in March, 1869," but it is not clear whether journal or separate was meant.

1870. "Ortalidinae Italicae . . . Pars VII—Fasc. 4." "Linea B. Tephritoidi." Pp. [1]-59. — As in Fascicle 3, the title and subtitle of the separate are on pp. [1] and [3] and the text begins on p. [5], corresponding to p. [5] of the Bullettino. Pages 31 and [105] of the Bullettino have been combined, by not repeating the title from [105], and the other pages remain identical in content. On p. [60] is a note that the fascicle is from the Bullettino, Vol. I (error for II!).

1870. (Title as in separate work). Bull. Soc. Ent. Ital. 2: [5]-31, [105]-133.

1871. "Ortalidinae Italicae . . ." (Fasc. 4, continued). Pp. [1]-53. — This continuation has a title page, as p. [1], the text beginning on p. [3] as in the corresponding part of the Bullettino. On p. [54] is a note that the article is from the Bullettino, Vol. III.

1871. (Title as in separate work). Bull. Soc. Ent. Ital. 3: [3]-24, [161]-188. — The title on p. [161] refers to "Fasc. 1," an obvious typographical error for Fasc. 4.

The text of the separate and journal works both begin on p. [3] and correspond through p. 24, the latter being a full page. For the remainder, the title, etc., on the top half of p. [161] of the Bullettino were dropped in the separate and all type moved forward a half page, thus preventing direct comparison of specific references between separate pages 25-53 and journal pages [161]-188.

Neave cites the Bullettino for Fascicle 4, and the Katalog cites the separate work. The Facsimile-Edition reproduces the journal articles.

[1871?]. Title page for completed Vol. VII of the separate works: "Species Italicae . . . descriptis" (as in title for Vol. VI, 1865). "Pars Sexta Scatophaginae Sciomyzinae Ortalidinae." "Dipter: Ital: Prodromi Pars [= Volume] VII." "1866-71."

The cover with this title page reads simply "Dipterologiae Italicae Prodromus A Camillo Rondani Vol. VII." It is presumed that cover and title page were supplied in 1871 after the last fascicle had appeared in separate printing. Sets of this separate Vol. VII were examined in the natural history museums at Vienna and London.

1877. "Species Italicae ordinis dipterorum (Muscaria Rnd)." "Stirpis XIX Sciomyzinarum revisio." Pp. [1] (title page) -78. — The title page also states that the work is an "Estratto dell' Annuario della Società dei Naturalisti di Modena Anno XI. — Fascicolo Primo." The text covers pp. [3]-75, with an Index on pp. [77]-78.

1877. (Title as in separate work). Ann. Soc. Nat. Modena, ser. 2, 11: 7-79. — This article must have appeared late in the year 1877. In the same issue are noted a number of works published in 1877 and received by the society. Near the end of the fascicle, on p. 126, is a death notice signed Aug. 1, 1877.

The slight difference in pagination between the separate and the journal article must have caused much wondering and confusion in the past. The separate also contains an Index, which the journal article does not have.

The Facsimile-Edition reproduces the 1877 work (from the journal), rather than the original (1868) work on the Sciomyzinae. Two new species, *Coremacera confluens* and *Sapromyza tabidiventris*, date only from this 1877 revision, although they are credited to the 1868 version by the Katalog (vol. 4, 1905) along with the others that rightfully take priority from 1868.

[1877?]. Title page for a Prodromus Vol. VII based on the journal articles (Sciomyzinae of 1877 version): "Species Italicae ordinis dipterorum ordinatim dispositae, methodo analitica distinctae, et novis vel minus cognitis descriptis." "Pars Sexta." "Stirpes XVIII-XX—Scatophaginae, Sciomyzinae, Ortalidinae." "Mediolani, Modenae, Florentiae 1867-1877." — I know this only from the Facsimile-Edition. The cover is the same form as that of the 1871 edition. On the inside of the cover is a list of the journal references.

PRODROMUS VOLUME VIII

1874. "Species Italicae ordinis dipterorum (Muscaria Rndn.) Stirps XXI. — Tanipezinae Rndn. collectae et observatae a Prof. Cammillo [sic] Rondani." Pp. [1]-16. — Page [1], with title and beginning of text, corresponds to p. 167 in the Bullettino. On p. 16, following the Index, is the imprint "Firenze, 1874," and the note "Dal Bull. Entomol., An. VI."

1874. (Title as in separate work.) Bull. Soc. Ent. Ital. 6: 167-182.

1874. "Species Italicae ordinis dipterorum (Muscaria Rndn.) collectae et observatae a Prof. Camillo Rondani." "Stirps XXII Loncheinae Rndn." Pp. [1]-32. — Page [1], with title and beginning of text, corresponds to p. 243 of the Bullettino. At the bottom of p. 32 is the imprint "Dal Bull. Entomol., An. VI., 1874. Firenze—Tip Cenniniana."

1874. (Title as in separate work.) Bull. Soc. Ent. Ital. 6: 243-274.

[1875]. (Title as in immediately preceding). "Stirps XXIII Agromyzinae." Pp. [1]-26. — Page [1], with title and beginning of text, corresponds to p. 166 of the Bullettino. Page [27] bears the note "Dal Bullettino Entomologico, Anno VII" midway on the page, and "Firenze, Tip. Cenniniana nelle Murate" in the lower left corner. No date is stated but Mik wrote "1875" in his copy, which I examined at Vienna.

1875. (Title as in separate work.) Bull. Soc. Ent. Ital. 7: 166-191.

1876. (Title as in preceding). "Stirps XXIV. Chylizinae Rndn." Pp. [1]-12. — Page [1], with title and beginning of text, corresponds to p. 187 in the Bullettino. At the bottom of p. 12 appears the imprint "Dal Bull. Entomol., An. VIII. 1876 Firenze—Tip. Cenniniana."

1876. (Title as in separate work.) Bull. Soc. Ent. Ital. 8: 187-198.

1880. (Title as in preceding, except for use of the abbreviation Rnd.). "Stirps XXV. Copromyzinae Zett." Pp. [1]-43. — Page [1], with title and beginning of text, corresponds to p. 3 in the Bullettino. Midway on p. [44] is the statement "Estratto dal fasc. 1° del Bullett. della Società Entomologica Italiana. Anno XII, pag. 3-45. Edito il 12 aprile 1880.—Firenze, Tipografia Cenniniana."

1880. (Title as in separate work.) Bull. Soc. Ent. Ital. 12: 3-45. — The imprint on the cover is "(Pubblicato il 12 aprile 1880)".

[1880?]. Cover and title page for a Prodromus Volume VIII, based on the journal articles listed above. I know these only from the Facsimile-Edition. The cover is the usual style for the Prodromus, stating that this is "Vol. VIII." On the inside of the cover are the journal references for the five stirpes composing the volume. The title is like that used for the 1877 edition of Anthomyinae in Vol. VI (see above): "Species Italicae . . . Pars Septima. Stirpes XXI-XXV—Tanipezinae, Loncheinae, Agromyzinae, Chylizinae, Copromyzinae." "Florentiae 1874-1880."

As with Volume VII, the Facsimile-Edition reproduces the journal articles. I have been unable to determine whether these pages were reproduced in 1880 as a complete volume, using the original page numbers, but this is of no practical consequence. For purposes of priority, the original articles take precedence.

The Anatomy of the Adult Male Reproduction System of *Aedes aegypti* (Linnaeus) (Diptera, Culicidae)[1]

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ABSTRACT

Re-investigation of the anatomy of the external reproductive system of adult male *Aedes aegypti* (Bangkok strain) shows, in addition to the already known facts, that (1) the 9th sternum is composed of anterior and posterior regions sharply divided from each other by the intersegmental membrane, (2) the basal paraproct (10th tergum) is attached to the dorsal root of the basimere and not to the lobe of the 9th tergum, and (3) the epiproct (remnant of the 11th tergum) is attached to the complex apical paraproct (10th sternum?). The present study of the internal genital system includes a description of the attachments of the apical filament of the testis and of the filaments of the accessory glands, and of the innervation of the different parts. It is suggested that the thin-walled tubes leading from the testes are the true vasa efferentia, and that the short, thicker-walled muscular region between them and the seminal vesicles represents the vasa deferentia. Males of 7 other species belonging to 4 genera of mosquitoes were dissected and compared with *Aedes aegypti*.

Although the anatomy of the external and internal reproductive system of male mosquitoes in general and of male *Aedes aegypti* in particular has received an extraordinary amount of attention (see Natvig 1948 and Christophers 1960), none of the numerous papers and monographs that we have examined gives an accurate version of all the anatomical components and their interrelationships for even a single species. The present paper is the result of a detailed re-investigation of the external and internal anatomy of the reproductive system in male adults of the Bangkok strain of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus). The findings on the internal anatomy are compared with the male reproductive system of seven other species of mosquitoes.

THE ANATOMY OF THE EXTERNAL REPRODUCTIVE SYSTEM

The swollen abdomen of the newly emerged adult *A. aegypti* male tapers posteriorly. The pale, grayish, unscaled, pleural membrane is conspicuous and tapers to the end of the eighth abdominal segment. The abdomen is composed of nine readily visible segments or uromeres. The first seven segments are roughly the same length but gradually decrease in diameter. The eighth segment is noticeably smaller than the seventh, and the ninth (or genital) segment is greatly reduced in size, although it gives rise to two very large and complex appendages. The tenth and eleventh abdominal segments (postgenital segments) are highly modified pieces embedded in the membranous region posterior to the ninth segment. In the individual mosquito the eighth through the eleventh abdominal segments constitute a *terminalium* (the plural term is *terminalia* as first used and correctly defined by Freeborn 1924; the plural term is often used as if it were a single noun). This definition takes into account the fact that the eighth abdominal segment undergoes rotation (Christophers 1915). Setae which become slightly longer and definitely more numerous posteriorly are present on the posterior margins of the first through the eighth terga and the first through the seventh sterna.

Since the terminalium in the male mosquito rotates 180° during the first day of adulthood, in the descriptions that will follow, the terms *dorsal* and *ventral* refer to structures as they are found in the newly emerged male with unrotated terminalium, unless specified to the contrary.

The ninth abdominal segment with its appendages (the parameres and aedeagus) constitute the *hypopygium* or *genitalium* (Snodgrass 1904, Crampton 1942). As in the case of many other insects, the ninth abdominal segment forms a complete ring about the abdomen (fig. 1). The ninth sternum is sometimes referred to as the *hypandrium* and the ninth tergum is termed the *epandrium* (Crampton 1942). A dorsal examination of the ninth segment of an intact living specimen shows only the large, sharply defined, dark brown, posteriorly pointed lobes of the ninth tergum (fig. 1, lnt). These dorsal lobes are thin and flat, and their outer margins curl inward. The ninth tergal plate itself (fig. 1, tp) is a thin, flattened, light brown structure retracted into the eighth segment. The ninth tergum abruptly narrows on each side to form a double ribbed, dark brown bar (fig. 1, lbt) that passes around the body. On the ventral surface the ninth tergal bar forms a large, dark brown, flat, posterior projection (fig. 1, pe); anteriorly the bar firmly fuses with the lighter brown ninth sternum as shown in figure 1. The ninth sternum is made up of (a) a large, pale brown, anterior portion (fig. 1, asp) which is completely obscured in the living specimen by the ventral intersegmental membrane, and (b) a small, narrow, darker brown, posterior portion (fig. 1, psp) which is embedded posteriorly in the conspicuous, membranous, fleshy, median, ventral lobe of the ninth segment

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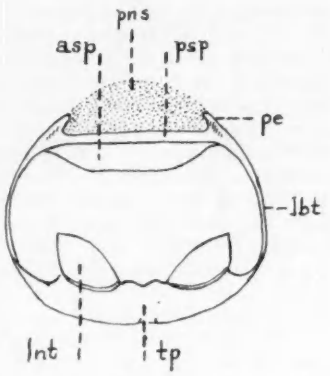
[3] Present address: 3790th Epidemiological Laboratory, USAF Aerospace Medical Center, Lackland Air Force Base, San Antonio, Texas. The opinions stated herein are the private ones of the author and are not to be considered the views of the United States Air Force.



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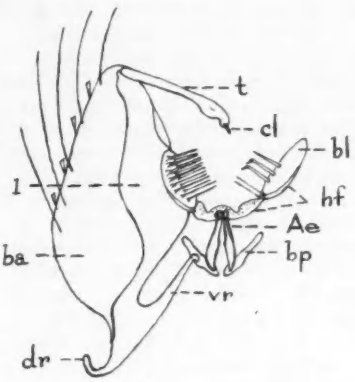

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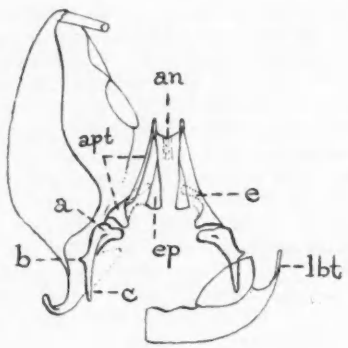

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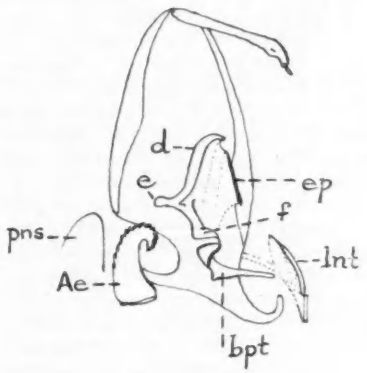


3B

FIG. 1.—Slightly schematic dorsal view of the ninth abdominal segment of *Aedes aegypti* male, showing the ninth tergal plate (tp), the lobe of the ninth tergum (lnt), the lateral bar of the ninth tergum (lbt), the posterior projection of the ninth tergal bar on the ventral surface (pe), the anterior portion of the ninth sternum (asp), the posterior portion of the ninth sternum (psp), and the membranous median lobe of the ninth segment (pns). Based on KOH-cleared whole mounts and dissections.

FIG. 2.—Schematic dorsal view of the appendages of the ninth abdominal segment of *A. aegypti* male, showing basimere (ba), telomere (t), terminal claw (cl), basal lobe of paramere (bl), harpagonal fold (hf), lacuna of paramere (1), dorsal root of basimere (dr), ventral root of basimere (vr), basal plate of aedeagus or endomere (bp), and aedeagus (Ae).

FIG. 3A.—Schematic dorsal view of the postgenital segment of *A. aegypti* male, showing ventrally folded distal end of the basal paraproct (a), spur of the basal paraproct (b) which attaches to the dorsal root of the basimere, the dorsal projection of the basal paraproct (c), the apical paraproct (apt), the anal opening (an), the epiproct (ep), and ventral projection of apical paraproct (e).

FIG. 3B.—Schematic lateral view of the postgenital segments of *A. aegypti* male, showing proximal portion of the apical paraproct (f), the ventral projection of the apical paraproct (e), the terminal, hooked portion of the apical paraproct (d), basal paraproct (bpt), and the epiproct (ep).

(fig. 1, pns). Only the anterior portion of the ninth sternum is illustrated by Christophers (1960, p. 461, fig. 60, 2, IX s"). The posterior portion of the ninth sternum is either very difficult or impossible to see clearly in KOH-cleared, flattened, whole mounted terminalia.

From the ventral portion of the ninth abdominal segment arise structures of considerable complexity. The two largest appendages have been variously referred to as *claspers* (Theobald 1907), *genital forceps*, or *gonostyles* (Crampton 1920), although it has been clear for some time that they are homologous to the parameres (literally side pieces) of other insects (Crampton 1942, Snodgrass 1959). Edwards (1920) suggested that these appendages were a specialized part of the ninth sternum, and Christophers (1922) subsequently showed that they arise as a pair of primary phallic lobes behind the ninth sternum of the larva. In the newly emerged adult male mosquito the large parameres are positioned ventrolaterally and are strongly attached to the abdomen dorsally by a dark brown basal apodeme (fig. 2, dr) which is inserted close to and ventral to the inward curving, lateral portion of the ninth tergal lobe. The first and largest segment of the paramere (fig. 2, ba) is termed either the *basistyle* (Edwards 1920), *side piece* (Christophers and Barraud 1923), *gonocoxite* (Christophers 1960), or *basimere* (Crampton 1942, Snodgrass 1957, 1959). Dorsal and ventral outward curving roots (or apodemes) of the basimere are deeply and complexly embedded within the ninth segment (fig. 2, vr, dr), where they serve as important sites of attachment for other appendages and muscles. The ventral and lateral portions of the basimere are dark brown and are covered with numerous, very fine hairs and are set with less numerous long, dorsally curving setae and scales. The inner, slightly concave face of the basimere, sometimes referred to as the *lacuna* (Freeborn 1924), is a clear, membranous region possessing numerous, very short, fine hairs (fig. 2, 1). The basal lobe of the paramere, on the ventral portion of the inner face of the basimere, is a large oval plaque which is slightly raised anteriorly (fig. 2, bl). Each basal lobe has 30 to 40 or more long, stiff, closely set hairs which slant dorsally and medianly so that the hairs from each lobe just touch each other posterior and dorsal to the anus. Usually two, but occasionally three, of the hairs on the ventral margin of the basal lobe are noticeably larger in diameter than the other hairs and their tips are bent about 80° so as to point anteriorly (see fig. 2). These specialized hairs were first illustrated by Ross and Roberts (1943, p. 43). In addition to those with bent tips, large straight hairs can be seen. The basal lobes attach anteriorly and laterally to small, thin, membranous, proximally located folds which in turn are connected in the midline with a median fold. This entire complex is the interbasal fold of Freeborn (1924) or the harpagonal fold of Christophers (1960) (fig. 2, hf), which, as Natvig (1948) so vividly describes it, is in the form of a ventral and lateral amphitheater about the aedeagus. The two straight, sharply pointed, medium-size, mesially projecting hairs on each of the lateral folds are termed the *harpago* by Christophers (1960).

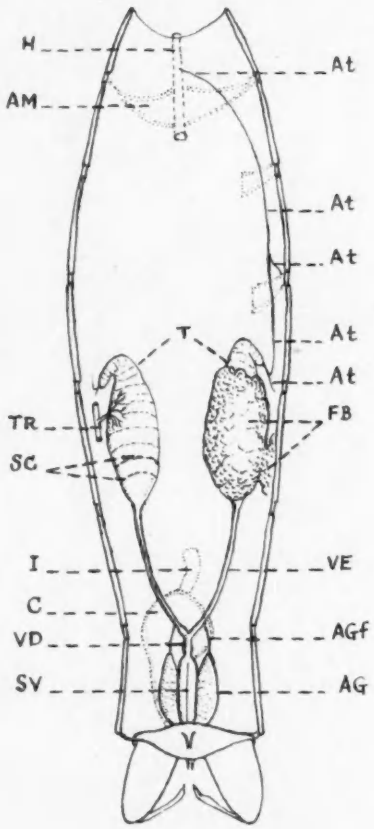
Near the distal end of the basimere is the second segment of the paramere. This segment is variously termed the *style* (Edwards 1932), *dististyle* or *distimere* (Crampton 1923), or *telomere* (Snodgrass 1957). Van Emden and Hennig (1956) have strongly objected to Crampton's (1942) use of the terms *paramere*, *basimere*, and *distimere*; but as Snodgrass (1957) points out their objections are not well founded. The style or telomere is a long, round, flexible, dorsally articulated, anteriorly projecting, brown structure which forms a rounded hump as it sharply narrows towards the distal end (fig. 2, t). In the intact, newly emerged male the telomeres converge so that their tips either touch or slightly cross in the dorsomedian line. The single, distinct, spinelike third segment inserted at the tip of the telomere (fig. 2, cl) is often referred to as either the *appendage of the dististyle* (Freeborn 1924) or the *claw* (Marshall 1938).

The two tiny, black, sclerotized pieces which are readily visible through the membranous, median, ventral lobe of the ninth segment of the living male (fig. 2, Ae) constitute the intromittent organ and are referred to as either the *aedeagus* (Edwards 1920, Snodgrass 1959) or *phallosome* (Freeborn 1924, Christophers 1960). The thin, ventrally converging, dorsally diverging plates of the aedeagus are large proximally and, as they narrow distally, are curved in a dorsal direction. Each plate is thicker ventrally than dorsally. On the distal half of the ventral ridge of each plate are approximately three rows of numerous small, black, sharp, posteriorly projecting spines or teeth (fig. 3, B, Ae). Edwards (1920) termed the spiny portion of the aedeagus the *mesosome*. The two plates of the aedeagus are connected at their distal ends by a thin dorsal bridge. Each plate articulates proximally with a thin, flattened, oblong, light brown sclerite (fig. 2, bp), which is the *endomere* of Crampton (1942) or the *basal plate* of Snodgrass (1959). The basal plates of the aedeagus have been incorrectly termed "parameres" by most culicidologists (see Christophers 1960, and Natvig 1948). The basal plate articulates at its center with the ventral root of the basimere (fig. 2).

The muscles of the hypopygium are well described and figured by Christophers (1960).

A large, fleshy, membranous anal lobe (the proctiger of Crampton 1920) opens anterior and ventral to the point where the basal lobe hairs touch in the median line; it is positioned somewhat ventrally, though the anal opening is still posterior and dorsal to the aedeagus in the newly emerged male. The longitudinal striations visible in the anal lobe of the living specimen are cuticular folds lining the rectum. The proctiger is supported by highly modified, twisted

sclerites termed paraprocts (literally beside the anus) by Freeborn (1924). Attached to the dorsal apodeme of the basimere by a short, pointed spur is the laterally positioned, dark brown basal paraproct (fig. 3 B, bpt). We believe the basal paraproct represents the tenth tergum (see also Snodgrass 1959, p. 69). The basal paraproct has a long, dorsally directed prong (fig. 3 A, c) which projects very close to but does not touch the ninth tergal plate or its lobe. This finding is not in agreement with Natvig (1948, p. 30), who states that the paraprocts articulate with the lobes of the ninth tergum. The basal paraproct has a short, blunt, ventrally directed lobe (fig. 3, A, a) which folds ventrally and then articulates with the long, yellow, complexly convoluted apical paraproct (fig. 3 A, apt). The apical paraproct possibly represents the tenth sternum (see also Edwards 1920). The proximal portion of the laterally positioned apical paraproct proceeds ventrally to form a short, median, ventrally directed projection (fig. 3 B, e); the paraproct then sharply folds dorsally, forming a long, laterally positioned, dorsally directed, terminally hooked projection (fig. 3 B, d). The apical paraprocts form the lateral sup-



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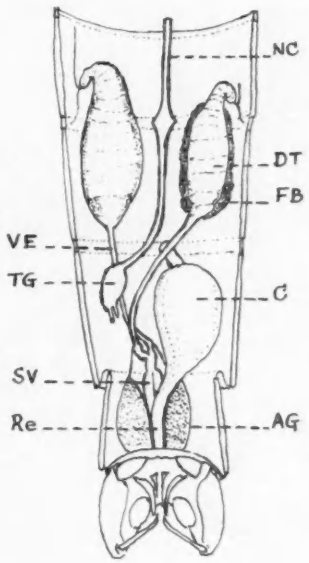

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FIG. 4.—Ventral view of the internal reproductive system of adult male *Aedes aegypti* before rotation of the terminalium, showing the heart (H), alary muscle (AM), apical testicular filament and its various branches (At), testis (T), testicular fat body (FB), trachea to testis (TR), testicular compartment (SC), vas efferens (VE), colon (C), vas deferens (VD), seminal vesicles (SV), accessory gland (AG), and accessory gland filament (AGf). Figure based on numerous in situ dissections. The colon is slightly displaced.

FIG. 5.—Ventral view of internal reproductive system of adult male *Aedes aegypti* following a counterclockwise rotation of the terminalium, showing abdominal nerve cord (NC), displaced testis (DT), terminal abdominal ganglion (TG), colon (C), vas efferens (VE), seminal vesicles (SV), accessory gland (AG), and rectum (Re). The colon is slightly displaced. The terminal abdominal ganglion is displaced anteriorly.

ports of the anal lobe and project posteriorly beyond it; Christophers (1960) labels them "cerci" and this is also Cole's (1927) interpretation. The ventral part of the apical paraproct is a thickened ridge, but the dorsal part of it forms a very thin sheet of colorless cuticle which connects with a thin, greatly flattened, light brown, triangular sclerite, the epiproct of Crampton (1918). The epiproct lies on the dorsal face of the anal cone (fig. 3 A, ep). Although this sclerite is said to represent the tenth tergum (Edwards 1920, Christophers 1960), we suggest that it may be the remnant of the eleventh abdominal segment.

THE ANATOMY OF THE INTERNAL REPRODUCTIVE SYSTEM

The testes of *A. aegypti* are typically located dorsolaterally in the sixth abdominal segment with their tips extending into the posterior margin of the fifth (fig. 4, T). In newly emerged males the testes may be so large that they extend from the sixth into the posterior margin of the fourth abdominal segment. Testes from old males are small and found only within the sixth segment; they definitely do not become swollen with age in adult life. We have never found the testes extending into the seventh segment. In newly emerged males the testes are normally of equal size and situated almost identically on opposite sides of the body. After rotation of the terminalium one testis is shifted into a more posterior position and is then noticeably smaller than the other in the same individual (fig. 5, DT).

In *A. aegypti* adults, a dense, variably thick, reddish brown-pigmented fat body firmly surrounds one-half or more of each testis, usually leaving the tip exposed (fig. 4, FB). The fat body is especially thick at the posterior end of the testes. If the seventh abdominal segment is slowly pulled into a drop of physiological saline (Ephrussi and Beadle 1936) while the adult is firmly held by the thorax, both testes can ordinarily be easily extracted from the abdomen. Occasionally during the extraction the fat body slips off the testes as a single piece. In general the fat body around the adult testis is tenacious and can be dissected away only with some difficulty, especially in older males; it is considerably less tenacious, however, in pre-imaginal stages of *A. aegypti*.

The wall of the testis of *A. aegypti* is a very thin and colorless sheath that completely envelops the organ. When the testes are examined in hematoxylin-stained whole mounts, the cellular structure of the tunic is obscure, although round or ovoid nuclei may be distinguished in it. In fresh, unstained whole mounts the nuclei are not usually visible. The testicular wall is not obviously double layered as it is in *Drosophila* (Miller 1950), except at the posterior end. The extremely thin strand of connective tissue attached to the anterior end of the testis is here termed the *apical filament* of the testis (fig. 3, At). In *A. aegypti* the apical filament divides into a posteriorly directed branch which attaches to the alary muscle between the fifth and sixth abdominal segments and into a more delicate anterior branch which extends to the second abdominal segment, where it inserts into the ventral wall of the heart. In some males an additional branch of the filament attaches to the alary muscle lying between the fourth and fifth abdominal segments.

The testes of newly emerged *A. aegypti* adults have three clearly defined regions: (a) a sharply demarcated, hyaline, laterally hooked, anterior zone; (b) a larger and longer, swollen middle zone; and (c) a large, gobletlike terminal zone. The middle and terminal zones have a distinct brownish cast with transmitted light, which is due to their internal contents and not to pigment, within the testicular sheath.

The extremely thin, nucleated sheath of the testis encloses a series of membranelike, individual compartments of variable dimensions containing germ cells in various stages of development (fig. 4, SC). In unstained whole mounts, the walls of the testicular compartments do not clearly reveal nuclei, but in hematoxylin-stained whole mounts nuclei can be seen. Although no muscle fibers or striations were observed on the testes in whole mounts stained with hematoxylin or Janus green B, or in unstained whole mounts examined with phase microscopy, portions of the testicular sheath on very rare occasions were seen to be capable of vigorous, rhythmic contractions in saline. In these cases, however, the testes were distinctly abnormal in shape.

During the first day of adulthood the terminal compartment of the testis opens into a long, straight, posteriorly and ventrally directed duct, here termed the *vas efferens* (fig. 4, VE; see Discussion). The vas efferens extends from the middle or posterior margin of the sixth to the middle or posterior margin of the seventh abdominal segment. The wall of the duct is noticeably thicker in newly emerged males than in older ones. Evenly spaced, lenticular nuclei are visible in the wall of the vas efferens in hematoxylin- or Janus green-stained whole mounts (fig. 6, VE), but additional cellular details are not clear. The uppermost portion of the vas efferens is usually dilated in mature males. Before rotation of the terminalium the vasa efferentia are taut and lie parallel to one another as they pass from the dorsolaterally located testes to the ventrally located seminal vesicles (fig. 4, VE). The ileum portion of the hind gut extends between the vasa efferentia. During rotation one vas efferens becomes twisted ventrally about the ileum in the direction of rotation of the terminalium, and the other vas is left slack and untwisted so that it passes dorsally from the unshifted testis to the dorsally rotated seminal vesicles (fig. 5). The vas efferens to the shifted testis is definitely shorter and either slightly or noticeably smaller in diameter than the other, and generally contains fewer spermatozoa. Material in the lumen of the untwisted vas efferens in older males often

has a pale sepia cast with transmitted light. No muscle fibers or striations were observed on the vasa efferentia in either unstained or stained preparations, and these tubes did not contract in saline whole mounts. Prashad (1916) referred to longitudinal muscles on the ducts leading from the testes in *Anopheles* [*maculatus*] *willmori* (James).

In the middle or near the end of the seventh abdominal segment, the relatively thin-walled vas efferens very abruptly joins a well-defined, short, thick-walled portion of the reproductive tract here termed the *vas deferens*, which has numerous, densely distributed, round nuclei each with a central nucleolus (fig. 6, VD). After a short distance these two thick-walled ducts join to form a common median duct located exactly ventral to the point where the ileum joins the saclike colon between the seventh and eighth abdominal segments (fig. 4, VD). The separated and united portions of the vasa deferentia both have a thin outer layer of circular muscle (fig. 6). In most dissections the vasa deferentia tend to be thrown into irregular folds. In a number of preparations the upper separated portions of the vasa deferentia were found to twitch rapidly and rhythmically (10 twitches in 4.0 to 7.8 seconds at 25° to 28° C.), especially in newly emerged males.

The common duct formed by the fusion of the vasa deferentia empties into two long, ventrally located seminal vesicles which are fused together within the eighth abdominal segment (fig. 4, SV). The two vesicles are conspicuously demarcated by what appears in stained and unstained whole mounts to be a single median division which separates them throughout their length. Only in histological sections is it apparent that this central division is actually double. During the first 24 hours after adult emergence, the single layer of cells making up the seminal vesicles is much more conspicuous in unstained whole mounts than later. During this period the cells are thick and hyaline, have granules bordering the lumen and appear to be secretory. The cells making up the seminal vesicles are typically larger than those of the vasa deferentia, are polygonal, and have a sharply defined, round nucleus with a centrally located nucleolus. When the seminal vesicles become filled with spermatozoa during the first day of adulthood, they have a distinct, pale sepia cast with transmitted light. With direct illumination, pale whitish masses of spermatozoa can be seen tightly aligned or sinuously coiled inside the vesicles. The vesicles, which are surrounded by a very thin sheath possessing very thin, widely spaced circular muscles, are capable of slight contractions in situ but do not contract in dissected whole mounts. Patches of fat body are scattered over the surface of the seminal vesicles, especially at the posterior end. Dorsally and on either side of the seminal vesicles are two large pear-shaped accessory glands which are approximately as long as the seminal vesicles (fig. 4, AG). A thin, conspicuous connective tissue filament is attached to the anterior end of each gland (fig. 6, AGf). The accessory gland filament has a single nucleus at the point of its attachment to the accessory gland sheath. Anteriorly the filament attaches precisely where the vas efferens joins the vas deferens (fig. 6). The accessory glands have a single, outer, richly nucleated sheath surrounding a single layer of large polygonal cells whose large round nuclei in whole mounts appear mostly peripherally located. The cells are filled with distinct, fine, round, acidophilic granules. The lumen in the accessory gland of the adult is not clearly visible in whole mounts, but it is readily visible in the accessory gland of the pupa. Like the seminal vesicles the accessory glands are capable of slight contrations in situ but they do not contract after dissection from the body. The very thin circular muscle fibers which encircle the glands can be seen clearly only in Janus green-stained whole mounts. In mature males freshly dissected, directly illuminated

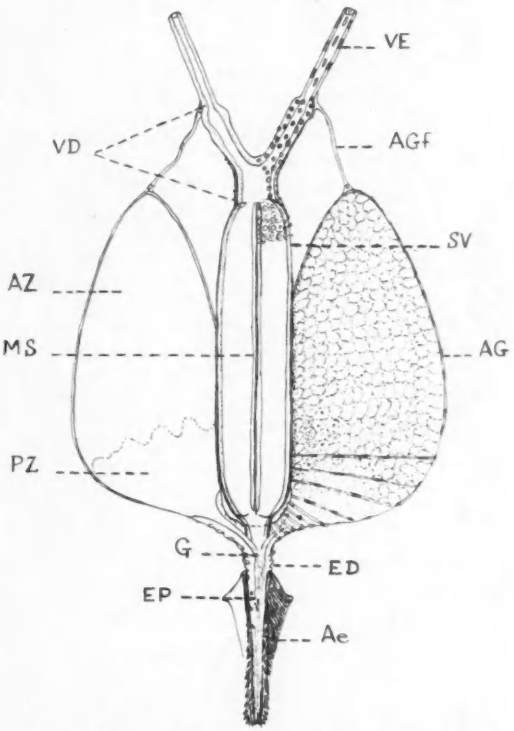


FIG. 6.—Semidiagrammatic ventral view of the posterior portion of the internal reproductive system of a newly emerged adult male *A. aegypti* showing details of the vas efferens (VE), seminal vesicles (SV), median division of the vesicles (MS), accessory glands (AG), accessory gland filament (AGf), anterior zone of accessory gland (AZ), posterior zone of accessory gland (PZ), region of the gonopore (G), ejaculatory duct (ED), endophallus (EP), and aedeagus (Ae). Figure based on unstained and stained whole mounts and histological sections. Note the thin circular muscles around the accessory glands, only a few of which are illustrated on the right gland.

glands usually have a large whitish anterior zone (fig. 6, AZ), irregularly demarcated from a clear, noticeably less dense, but granular posterior zone (fig. 6, PZ). Circular muscles can be seen around the outer wall of the posterior efferent duct of the accessory gland (fig. 6). Although the walls of the ducts unite dorsally before emptying into the anterior end of the ventral ejaculatory duct, the cuticle-lined lumina do not unite but separately open at the gonopore (fig. 6). Although the accessory glands increase in size during the first 24 hours of adult life (table 1), this increase is strikingly less than their rapid growth during the pupal stage.

Table 1.—Changes in the size of the accessory glands of *Aedes aegypti* (L.) during the first day of adulthood.

Condition of terminalia	No. adults	No. glands measured	Average dimensions in millimeters	
			Length	Width
Unrotated	8	15	0.31	0.12
Rotating	3	6	0.34	0.12
Rotated	4	8	0.35	0.14

The invagination which forms the short ejaculatory duct is well formed in early pupal life, but its cuticle-lined lumen does not open into either the seminal vesicles or the accessory glands at this time. The disc which forms the aedeagus is not fully differentiated in the early pupa but subsequently it elongates and as it does so the lumen inside it forms the endophallus. The endophallus extends to the posterior tip of the aedeagus where a small opening (the phallotreme) is found. Kulagin (1907, p. 874) thought that the endophallus of *Culex pipiens* Linnaeus terminated at the center of the aedeagus. In the old pupa the endophallus opens anteriorly into and becomes continuous with the ejaculatory duct, and the ejaculatory duct opens into the seminal vesicles and accessory glands. In the newly emerged adult the lumen of both endophallus (fig. 6, EP) and ejaculatory duct (fig. 6, ED) contain distinct longitudinal cuticular folds. Round to ovoid nuclei are visible and only a few poorly defined muscles can be seen in whole mounts of endophallus and ejaculatory duct.

The terminal abdominal ganglion of the adult, which is strikingly larger than any of the other abdominal ganglia, represents the fused seventh and eighth abdominal ganglia of the larva, as Hurst showed (1890). This ganglion lies at the posterior end of the seventh abdominal segment (in some preparations between the seventh and eighth segments). A small, anteriorly directed trunk (fig. 7, N-1) sends nerves laterally into the seventh abdominal segment. A separate, more posteriorly located trunk (fig. 7, N-2) sends nerves posterolaterally into the eighth abdominal segment. Large complex terminal trunks of the ganglion (fig. 7, N-3) extend dorsally and posteriorly between the vasa deferentia.

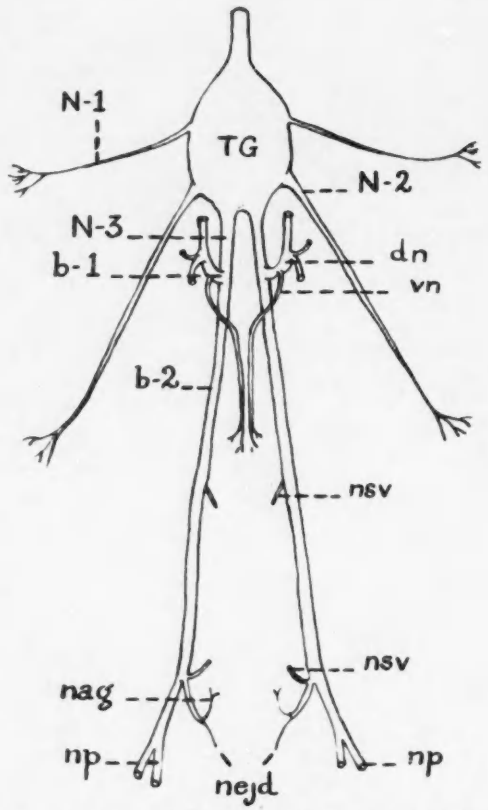


FIG. 7.—Schematic view of the terminal abdominal ganglion (TG) and its principal nerves: N-1, anterior nerve trunk; N-2, posteriorly located nerve trunk; N-3, terminal nerve trunk; b-1, nerve branch from terminal trunk which innervates the dorsal colon; dn, dorsally directed nerve branching from terminal nerve; vn, nerve branch from terminal trunk which innervates the ventral skeletal muscles of the eighth segment; b-2, nerve branch from terminal trunk which innervates seminal vesicles (nsv), accessory glands (nag), ejaculatory duct (nejd), and parameres (np).

Each of these terminal nerve trunks bifurcates into two main branches. The first branch (fig. 7, b-1) divides into a dorsal nerve (dn) that innervates the colon and a ventral nerve (vn) that innervates the skeletal muscles of the eighth sternum. The second main branch (fig. 7, b-2) of the terminal trunk extends between the seminal vesicles and the accessory glands, and separately innervates both of these structures (nsv, nag) as well as the ejaculatory duct (nejd). The second main branch (b-2) bifurcates just before entering the parameres (np). After rotation the terminal abdominal ganglion is shifted to a ventrolateral position.

SOME MISCELLANEOUS ANATOMICAL OBSERVATIONS ON SEVEN OTHER MOSQUITOES

To supplement the work with *A. aegypti*, general

observations on the internal anatomy of the male reproductive system were made on the following mosquitoes: *Anopheles quadrimaculatus* Say (U.S. Naval Medical Research Institute strain) (fig. 8), *Anopheles gambiae* Giles (Ross Institute strain) (fig. 9), *Culex pipiens pipiens* Linnaeus (U.S. Naval Medical Research Institute strain) (fig. 10), *Culex p. quinquefasciatus* Say (Walter Reed strain from Malaya) (fig. 11), *Culex tritaeniorhynchus* Giles (Walter Reed strain from Japan) (fig. 12), *Haemogogus equinus* Theobald (National Institutes of Health strain) (fig. 13), and *Psorophora howardii* Coquillett (Vero Beach, Florida, strain) (fig. 14).

In *Anopheles quadrimaculatus* an apical testicular filament is visible and the testes are ovoid to pear-shaped. While the anteriormost region of the testis is colorless, the contents of the posterior region appear yellowish brown with transmitted light and whitish when directly illuminated. The finely and evenly wrinkled, thin-walled duct of each vas efferens abruptly joins a thick-walled, posteriorly enlarged duct. That portion of the thick-walled duct which is anterior to the uppermost attachment of the accessory glands is believed to be the vas deferens. Striations are visible in the walls of this region, and in some preparations this particular portion of the reproductive tract vigorously contracted. Even so, the vas deferens is not a sharply defined anatomical region in the *Anopheles* examined. The more extensive remaining portion of the duct is the seminal vesicle, which is firmly attached along its entire dorsolateral aspect to the round accessory glands. Connective tissue between the accessory glands and the seminal vesicles is most clearly visible in whole mounts at the anterior end of the glands. A few strands of connective tissue can sometimes be observed stretching between the separated vesicles (fig. 8, Ct). The accessory glands which appear whitish with direct illumination are dark with transmitted light. Each gland abruptly empties (a discrete accessory gland duct could not be seen in whole mounts) at its equator laterally, along with the seminal vesicles, into the large ejaculatory duct. The ejaculatory duct is surrounded by more than 20 large, well-tracheated, circular muscles. This duct was observed to contract slowly in a vermiform manner in saline whole mounts. In some dissections a colorless cuticular tube continuous with and posteriorly projecting from the lumen of the ejaculatory duct was seen (fig. 8, Ce, endophallus?).

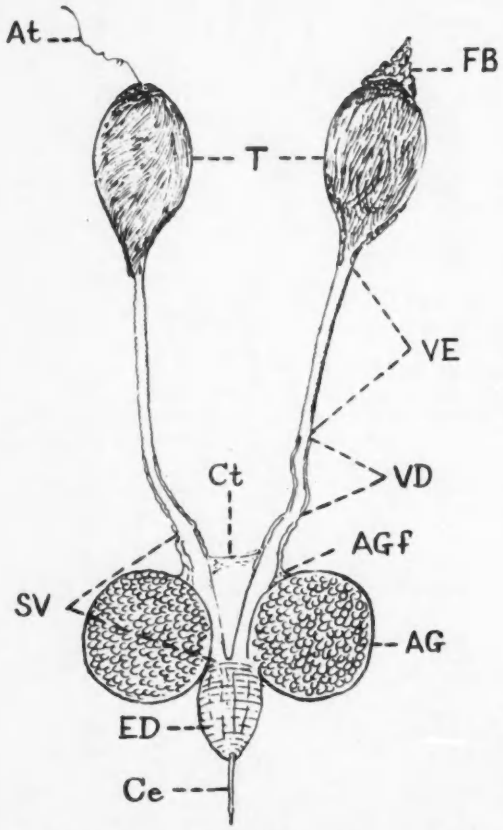


FIG. 8.—Internal reproductive system of male *Anopheles quadrimaculatus*, showing apical filament of testis (At), testis (T), fat body (FB), vas efferens (VE), vas deferens (VD), seminal vesicles (SV), accessory glands (AG), and ejaculatory duct (ED). The cuticular tube that extends from the end of the ejaculatory duct (Ce) passes through the aedeagus. In some preparations strands of connective tissue (Ct) were observed between the seminal vesicles. The accessory glands are firmly fused to the seminal vesicles. The connective tissue attaching the accessory glands to the vesicles is most conspicuous anteriorly (AGf).

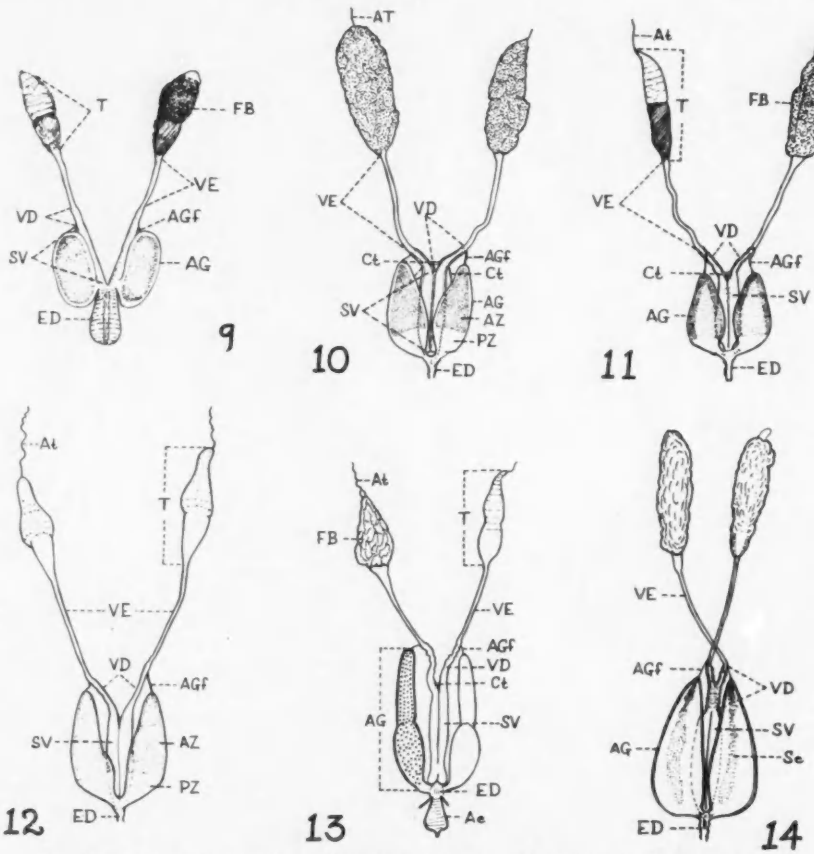
The male reproductive system of *Anopheles gambiae* (fig. 9) appears generally similar to that of *A. quadrimaculatus* except for two points: (1) the testicular sheath in *A. gambiae* is uniformly dark brown with both direct and transmitted light, and (2) the accessory glands are ovoid, have clear edges, and are filled with pale, lemon-yellow granules.

In the three species of *Culex* (figs. 10, 11, 12) studied an apical testicular filament is present (the longest and most conspicuous filament being found in *C. tritaeniorhynchus* (fig. 12).) The pear-shaped, anteriorly hooked testes in the *Culex* examined are entirely and densely covered with brown pigmented fat body. With transmitted light the posterior portion of the testis is brownish, the anterior part colorless. The vas efferens in *Culex* is the long, thin-walled duct proceeding from the posteriormost end of the testis to the anterior attachment of the accessory gland filament. It is suggested that the vas deferens is the thicker walled region which extends posteriorly from the attachment of the accessory gland filament to the point where it externally fuses with the other duct in the median ventral line to form the seminal vesicles. The median longitudinal division in the seminal vesicles is distinctly double in *Culex*. Connective tissue filaments between the

vesicles are conspicuous in *C. pipiens* (fig. 10, Ct) and *C. quinquefasciatus* (fig. 11). The seminal vesicles discharge posteriorly into a short common duct. The large, pear-shaped accessory glands in the three species possess a large, golden to greenish-yellow anterior zone sharply and regularly demarcated from a smaller, colorless posterior zone (figs. 10, 11, 12, PZ, AZ). The yellow material in the accessory glands rapidly decolorizes in ethanol. The short posterior ducts of the accessory glands fuse dorsally into a common median duct before emptying into the short ejaculatory duct.

With the exception of the accessory glands the internal anatomy of the male reproductive system of *Haemogogus equinus* (fig. 13) generally resembles that of *Culex*. The whitish to colorless accessory glands of *H. equinus* differ strikingly from those of the other mosquitoes examined in this study in being divided into an ovoid posterior part and a longer, narrower, vermiform anterior part. The glands are enclosed in a sheath with circular muscles.

The elongated testes of *Psorophora howardii* are entirely and densely covered with fat-body tissue. The vasa efferentia empty posteriorly into short muscular vasa deferentia which fuse into a heavily musculated, vigorously contractile common duct before emptying into the two large seminal vesicles. The pear-shaped accessory glands in a young virgin male contain dark yellow material at the apex. This yellow secretion inside the glands became lighter yellow in the lower part of the glands. The accessory glands of *P. howardii* behaved quite differently from those of the other mosquitoes used in this study in that they rhythmically contracted (mostly in a posterior direction) in saline whole mounts (10 contractions in 5.6 to 6.6 seconds). The acces-



FIGS. 9 THROUGH 14.—The internal reproductive system of adult males of various mosquitoes; *Anopheles gambiae* (FIG. 9), *Culex pipiens* (FIG. 10), *C. quinquefasciatus* (FIG. 11), *C. taeniorhynchus* (FIG. 12), *Haemogogus equinus* (FIG. 13), and *Psorophora howardii* (FIG. 14), showing accessory glands (AG), accessory gland filament (AGf), apical filament of testis (At), anterior zone of accessory gland (AZ), connective tissue between seminal vesicles (Ct), ejaculatory duct (ED), testicular fat body (FB), posterior zone of accessory gland (PZ), secretion in accessory gland (Se), seminal vesicles (SV), testes (T), vas deferens (VD), vas efferens (VE), and aedeagus (Ae).

sory gland sheath appeared essentially the same as in the other mosquitoes.

COMPARISON OF SPECIES

It is now evident that adult male mosquitoes in general have (a) an apical testicular filament, (b) ovoid, elongated, or pear-shaped testes internally divided into separate compartments, (c) a usually dense, pigmented fat body jacket, (d) a thin-walled, well defined vas efferens, (e) a short, thick-walled, often poorly defined, but usually muscular vas deferens, (f) seminal vesicles which are either completely separated or fused through part or all of their length, (g) laterally positioned, round, ovoid, or elongated accessory glands sometimes filled with yellow material, and (h) a variably developed, muscular ejaculatory duct, and (i) an endophallus.

Anopheles males examined in this study differ principally from the other species studied in (a) having the seminal vesicles separate throughout their length, (b) having the accessory glands fused along the entire dorsolateral surface of the vesicles, and (c) having a very large, heavily musculated ejaculatory duct. The vas deferens is less clearly defined in *Anopheles* than in males of the other genera.

In males of the other genera studied, the seminal vesicles are fused, the accessory glands completely separated from the seminal vesicles, and the ejaculatory ducts are poorly developed.

Yellow material is seen in the accessory glands of *A. gambiae*, in all three species of *Culex*, and in *Psorophora howardii*.

The vasa deferentia are most clearly defined in *Aedes* and *Psorophora* species, and fuse into a common duct before emptying into the seminal vesicles.

Haemogogus equinus males differ from those in the other genera of mosquitoes studied by having the accessory glands divided into two distinct but connected regions.

DISCUSSION

With regard to the external reproductive system we have followed Snodgrass (1957, 1959) in using those terms which differ from the nomenclature employed by most culicidologists [*basimere* rather than *basistyle*, *telomere* rather than *dististyle*, *aedeagus* rather than *phallosome* or *mesosome*, and *basal plate* or *endomere* (Crampton 1942) rather than *paramere*]. The term *paramere* as used by culicidologists (e.g., Freeborn 1924, Natvig 1948, and Christophers 1960) refers to a structure that is not homologous with the forcepslike appendages of the ninth abdominal segment of other insects (Snodgrass 1957). The clasping organs are the true parameres of other insects, as Snodgrass (1957) has pointed out.

As far as we are aware, the relation between the basal paraproct and the hypopygium in mosquitoes has either been ignored or inaccurately shown by other workers; the belief that the paraproct attaches to the lobe of the ninth tergum (Natvig 1948) is clearly not true for *A. aegypti*. Its attachment to the dorsal root of the basimere, as shown in this study, is in harmony with its behavior during copulation (Wheeler and Jones 1960).

The testes of most, if not all, mosquitoes are typically found in the sixth abdominal segment, as Hurst (1890) first showed with *Culex*. They possess an apical filament, as Imms (1908) first showed with *Anopheles maculipennis* larvae, and are partially or entirely encased in a dense, pigmented fat body, as Cholodkovsky (1905) first showed with *Culex*. Although Kulagin (1907), Lomen (1914), and Langeron (1926) thought of this fat body as a distinct outer layer formed by the testis itself, it does not differ histologically from the fat body seen elsewhere in the abdomen, and indeed it can be seen to arise from it during larval and pupal life. The fat body around the testis is special in the sense that Christophers (1960) uses it in the fact that the testis is the only organ in the adult body so thickly and firmly encased. That the fat body often slips off the testis during dissections is additional proof it is not produced by the testis.

The testes of various mosquitoes have been shown by many investigators to be oval, elongated, or pear-shaped structures divided into compartments termed spermacysts (Cholodkovsky 1905, Kulagin 1907, Lomen 1914, Christophers 1960). Although the testicular sheath in many Diptera is itself pigmented (Keuchenius 1913, Patton and Cragg 1913, Hori 1960), this is generally not true for mosquitoes (*A. gambiae* is an exception to the rule). While the posterior portion of the testis, vas efferens, vas deferens, and seminal vesicles in most mosquitoes appear pale brown with transmitted light, these same portions are essentially whitish when directly illuminated.

Although the duct that proceeds posteriorly from the testes of mosquitoes is generally termed the *vas deferens*, beginning with Dufour (1851), we believe this term is incorrect because, as in all other Diptera (see Cholodkovsky 1905), their testis is but a single sperm tubule or follicle (as Whiting 1917, and Snodgrass 1959, have recalled), and therefore its mesodermal exit duct on strictly anatomical grounds should be termed the *vas efferens* (see also Snodgrass 1935, p. 570, fig. 293 A, Ve). Histologically these tubes resemble the testicular sheath. The studies of Hurst (1890) and Christophers (1922, 1960) show that the tissue from which the true vas deferens differentiates arises quite separately from the long mesodermal strand (or vas efferens) seen on the larval testis, and that later the two ducts grow together. In some Diptera the vas efferens is said to atrophy in the pupal or adult stage and to be replaced by the ectodermally derived vas deferens (Friele 1930, Abul-Nasr 1950). In some other Diptera (*Calliphora* and *Drosophila*) the larval testes do not possess a posterior mesodermal tube and the genital ducts arise mostly independently of the gonad from an imaginal disc (Brüel 1897, Dobzhansky 1930, Stern and Hadorn 1939). In

these insects, then, the formation of the vas efferens is nearly entirely suppressed (see Miller 1941).

In mosquitoes there is no evidence that the vas efferens degenerates and is secondarily replaced by the vas deferens. Indeed, the vas deferens appears to have been very nearly suppressed in *Anopheles*, is slightly better developed in *Culex* and *Haemogogus*, and, though well formed in *Aedes* and *Psorophora*, is considerably shorter than the vas efferens. It should be mentioned, however, that both Kulagin (1907) and Perfiliev (1930) considered the entire system posterior to the testis to be vas deferens—i.e., ectodermally derived.

Although several workers have illustrated and referred to the seminal vesicles in *Culex* (Prashad 1916) and *Aedes* (Prashad 1916, Hayes 1953) as an enlarged, median "common sperm duct," this is in error; for, as indicated in this study and in that of Lum (1961a), the seminal vesicles are two distinct tubes (whether outwardly joined or not) in representative *Anopheles, Deinocerites, Culex, Culiseta, Aedes, Haemogogus, Psorophora,* and *Uranotaenia.*

The accessory glands of mosquitoes vary considerably in color and shape. Colorless, white, and varying shades of yellow material have been reported by Lum (1961b). Round or ovoid glands have been described for various *Anopheles* (Perfiliev 1930, Lum 1961a). Lum (1961a) found pear-shaped glands in *Uranotaenia lowii* Theobald, *Psorophora howardii, Culex quinquefasciatus,* and *Aedes taeniorhynchus* Wiedemann; and he referred to elongated glands in *Culiseta inornata* (Williston), *Aedes infirmatus* Dyar and Knab, *Aedes fulvus pallens* Ross, and *Aedes triseriatus* (Say), and to elongated, centrally constricted glands in *Deinocerites cancer* Theobald. The anterior filament of the accessory glands was probably first seen by Eysell (1905, fig. 5), although he mistook it for the efferent duct of the gland. Burcham (1957) was the first to give a really accurate account of the filament, in *A. aegypti.* Lum (1961) has recently described accessory gland filaments in many mosquitoes but did not observe them in either *Anopheles crucians* Wiedemann or *Uranotaenia lowii.* In the present study accessory gland filaments were observed in all species examined.

The short lateral and median common ducts of the accessory glands in all of the mosquitoes except *Anopheles* appear to have been generally overlooked. Snodgrass (1959, his fig. 30 D and E, Dej) illustrated them in *Aedes aegypti* and *Culex quinquefasciatus* but mislabelled the common median accessory gland duct as the ductus ejaculatorius.

While the ejaculatory duct is a large, heavily muscular organ in various *Anopheles* as Perfiliev (1930) first showed, in the other genera of mosquitoes the duct is comparatively poorly developed, as Prashad (1916) has shown for *Aedes, Culex,* and *Culiseta,* and as Lum (1961) has shown for *Deinocerites, Uranotaenia,* and *Psorophora.*

SUMMARY

Unless specified differently, the terms *dorsal* and *ventral* refer to structures as they are found in the newly emerged, adult male with unrotated terminalia, of the Bangkok strain of *Aedes aegypti.*

1. The terminalium consists of the pregenital eighth abdominal segment, the genital ninth segment with its appendages (hypopygium), and the highly modified, postgenital tenth and eleventh abdominal segments (proctiger).

2. The appendages of the ninth segments are the large, external, forcepslike parameres (=claspers) and the small, internal aedeagus (or phallosome). Each paramere is composed of a large, complex basimere, a slender telomere (=style), and a small terminal claw. The aedeagus is fashioned from two thin, flattened, dorsally curved, sclerotized plates held together at the distal end by a thin dorsal bridge. At the proximal end the plates of the aedeagus fasten to endomeres or basal plates which articulate with the ventral root of each basimere.

3. The tenth abdominal segment is supported on each side by two highly modified sclerites termed the paraprocts. The basal paraproct (tenth tergum) attaches proximally to the dorsal root of the basimere and distally to the apical paraproct (tenth sternum). The tips of the apical paraproct extend beyond the anal opening. The epiproct, which is believed to be a remnant of the eleventh abdominal segment, is attached to the apical paraproct and lies on the dorsal face of the membranous anal cone.

4. The pear-shaped, dorsolaterally located testes may extend from the posterior end of the sixth to the posterior margin of the fourth abdominal segment, though they are often found only within the sixth. They are never located in the seventh abdominal segment. In the newly emerged male they are symmetrically located, but after rotation of the terminalium one of them is shifted to a more posterior position. In the rotated male the shifted testis is smaller than the other one.

5. The nucleated sheath surrounding the testis is colorless. Internally the testis is divided into separate compartments. A mass of brown pigmented fat body encloses one-half or more of the testis and is especially thick at the posterior end of the organ.

6. The apical filament of the testis usually divides into two delicate branches which are variously inserted into alary muscles and heart.

7. The testes open posteriorly into two long, ventrally directed, thin-walled ducts which are the vasa efferentia. In the seventh abdominal segment these ducts join two short, muscular ducts which are the vasa deferentia.

8. The vasa deferentia fuse into a median, common duct before emptying into two long, ventral, seminal vesicles which are fused together in the eighth abdominal segment.

9. Dorsolateral to the seminal vesicles are large,

pear-shaped accessory glands which empty into a short median, poorly musculated duct that then penetrates the aedeagus.

10. The terminal abdominal ganglion sends separate nerves to (a) skeletal muscles of the seventh abdominal segment, (b) skeletal muscles of the eighth abdominal segment, (c) the colon, and (d) the seminal vesicles, accessory glands, ejaculatory duct, and parameres.

11. The internal reproductive system of *Anopheles quadrimaculatus* and *A. gambiae* males differs from that of *A. aegypti* in having poorly defined vasa deferentia, separated seminal vesicles, round or ovoid accessory glands, and a very large muscular ejaculatory duct.

12. The internal reproductive system of *Culex pipiens, C. quinquefasciatus,* and *C. tritaeniorhynchus* males principally differ from *A. aegypti* in not having the vasa deferentia fused posteriorly into a common duct.

13. *Haemogogus equinus* males differ from the other species examined in having the accessory glands divided into an ovoid posterior part, and a long, vermiform anterior part.

14. The internal reproductive system of male *Psorophora howardii* resembles *A. aegypti* in having well-defined separated vasa deferentia which posteriorly fuse into a common duct before emptying into the seminal vesicles.

ACKNOWLEDGMENTS

Ronald E. Wheeler has greatly contributed to our knowledge of the external reproductive anatomy of *A. aegypti* by calling to our attention many facts which would otherwise have escaped notice.

We have been most fortunate indeed in having the advice of Dr. Robert E. Snodgrass and Sir Rickard Christophers, and we wish to thank them for having critically read the manuscript. The manuscript was additionally improved by the comments of Drs. Ralph Barr, W. E. Bickley, P. T. M. Lum, and Ernestine B. Thurman. Dr. Bettina Rosay made numerous helpful comments and suggestions for which we are especially grateful.

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We are particularly indebted to Dr. George Davidson of the Ross Institute for making *Anopheles gambiae* available to us.

ADDENDUM

Some authors consider the "basal lobe" of Christophers to be the "claspette lobe" because of its ventral location (Thurman, E. H. B. 1959. A contribution to a revision of the Culicidae of Northern Thailand. Univ. Md. Agric. Expt. Sta. Bull. A-100, 182 pp.).

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Observations on the Subgenus *Argas* (Ixodoidea, Argasidae, *Argas*). 4. *A. neghmei*, New Species, from Poultry Houses and Human Habitations in Northern Chile[1]

GLEN M. KOHLS[2] AND HARRY HOOGSTRAAL[3]

ABSTRACT

Argasid ticks occurring in chicken houses, dovecotes, and human habitations at Calama and Chuquicamata, Province of Antofagasta, in northern Chile, have previously been identified by some workers as *Argas persicus* (Oken) and by others as probably a local variant of *A. reflexus reflexus*. Present studies, especially of the larvae, indicate that the Chilean ticks represent a new species of the *reflexus* (F.) group for which the name *A.* **neghmei** is proposed. Adults, nymphs, and larvae are described.

Early in 1950, Dr. Amador Neghme of the Department of Parasitology, University of Chile, Santiago, sent several adults and nymphs of an argasid tick to the Rocky Mountain Laboratory for identification. The specimens had been collected in February 1949, in chicken houses, dovecotes and human habitations in Chuquicamata and Calama, Province of Antofagasta, in northern Chile. According to Porter (1928), during April of that year, large numbers of poor people living in this area had been severely attacked at night by ticks which were present in great abundance and which he and Dr. F. Lahille of Buenos Aires identified as *Argas persicus*. Adult specimens collected earlier at Calama had also been identified by Lahille (1915) as this species. Although the specimens received from Dr. Neghme resembled *A. persicus* (Oken) in certain respects, they were clearly not that species but instead were more nearly allied to *A. r. reflexus* (Fabr.) of Europe. They were identified by Kohls as probably local variants of that species (see Hoogstraal 1956, p. 77), but further study, especially of the few larvae obtained

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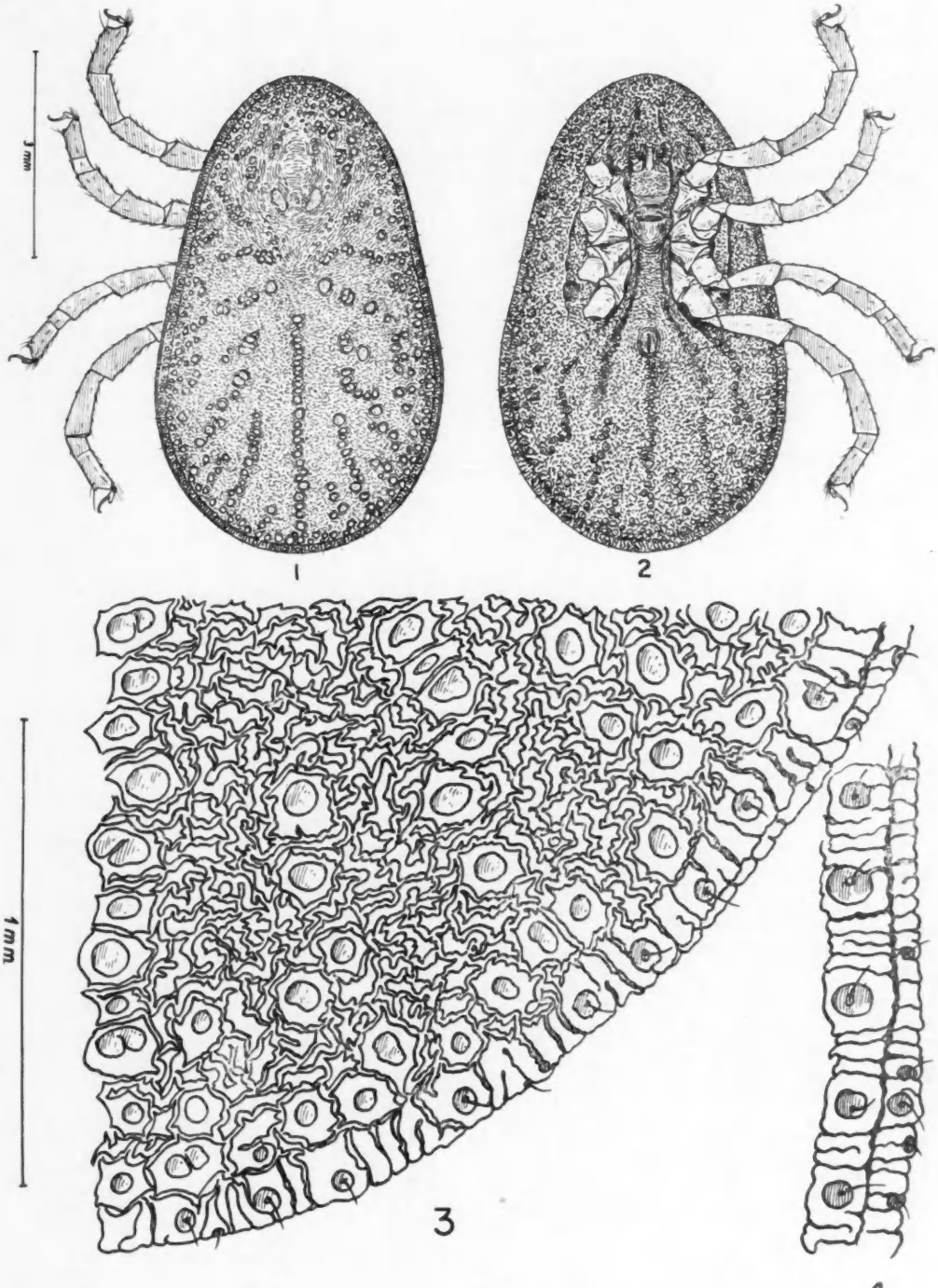


Argas neghmei, n. sp., adult female paratype from Chuquicamata, Chile. FIG. 1.—Dorsal view. FIG. 2.—Ventral view. FIG. 3.—Integument, posterior dorsal quadrant. FIG. 4.—Integument, lateral view.

from one of the females, indicates that the Chilean specimens are a new species. We take pleasure in naming the species for Dr. Neghme in recognition of his many contributions to knowledge in the field of parasitology. Dr. Neghme informs us that the ticks are known locally as "paltas."

Argas (Argas) neghmei, new species

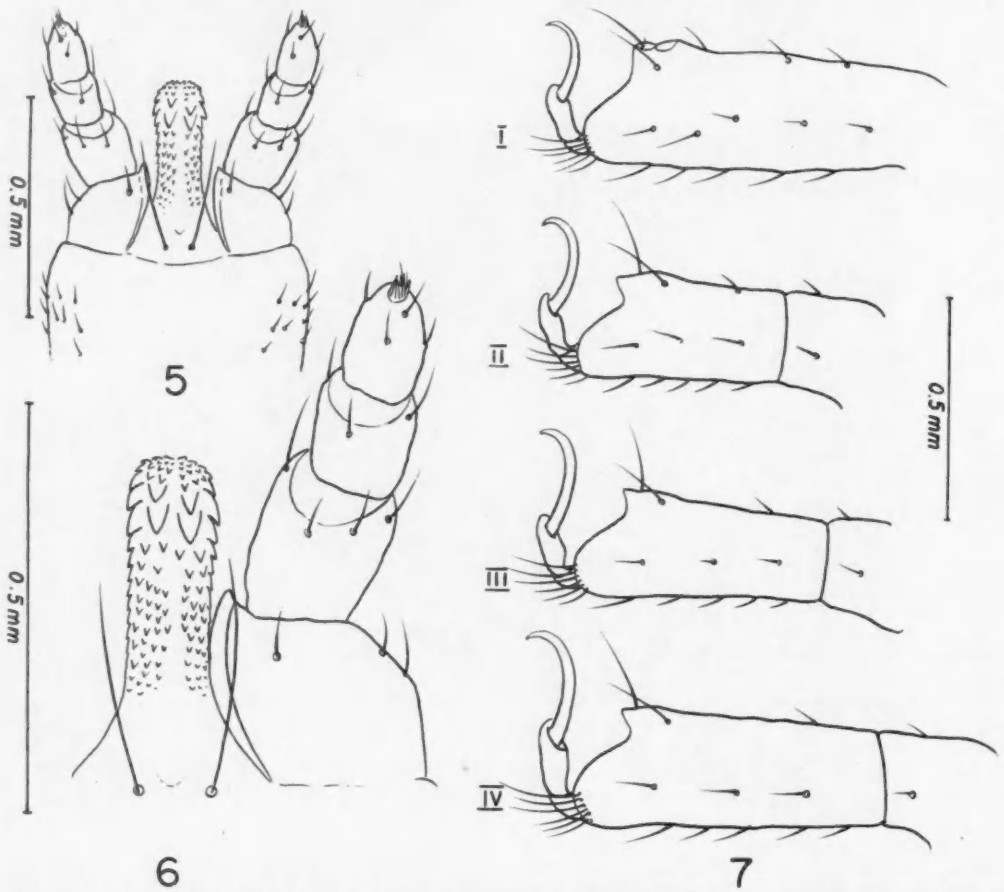
Neghme's South American Bird Argasid

(Figures 1-7)

FEMALE (figs. 1-7).—Measures from 5.53 mm. to 10.76 mm. in length and from 3.28 mm. to 6.45 mm. in width. The holotype, an average specimen, measures 8.20 mm. in length and 5.12 mm. in width. Body (unengorged) thin and flat, characteristic of *A. reflexus* group; outline moderately elongate with bluntly rounded posterior margin, comparatively narrowly rounded anterior margin, and gradually converging lateral margins.

Dorsal integument posteriorly (fig. 3) with elevations consisting of small, curved and undulating ridges of various lengths and widths. Anteriorly, elevations are somewhat smoother and form a definite pattern around discs. *Setae* fine, inconspicuous, widely spaced, arising from minute pits in dorsal and lateral integument. *Discs* large and distinct, arranged in clearly radiating rows (fig. 1). *Lateral integument* divided by a continuous suture (fig. 4); dorsolateral surface with quadrangular or subquadrangular *persicus*-like "cells," each bearing a small seta and each, except some of those anteriorly, is separated from the other by a strip of striated *reflexus*-like integument of varying width as figured; ventrolateral surface with irregular *reflexus*-like striations, among which small setae arise and a few of which are wide enough to suggest "cells." *Ventral integument* (fig. 2) quite similar to that of dorsal surface; discs arranged radially as illustrated; a small inconspicuous patch of longer, pale setae arising anterior of camerostome. *Hood* absent.

Capitulum (figs. 5-6) moderate in size. *Basis*



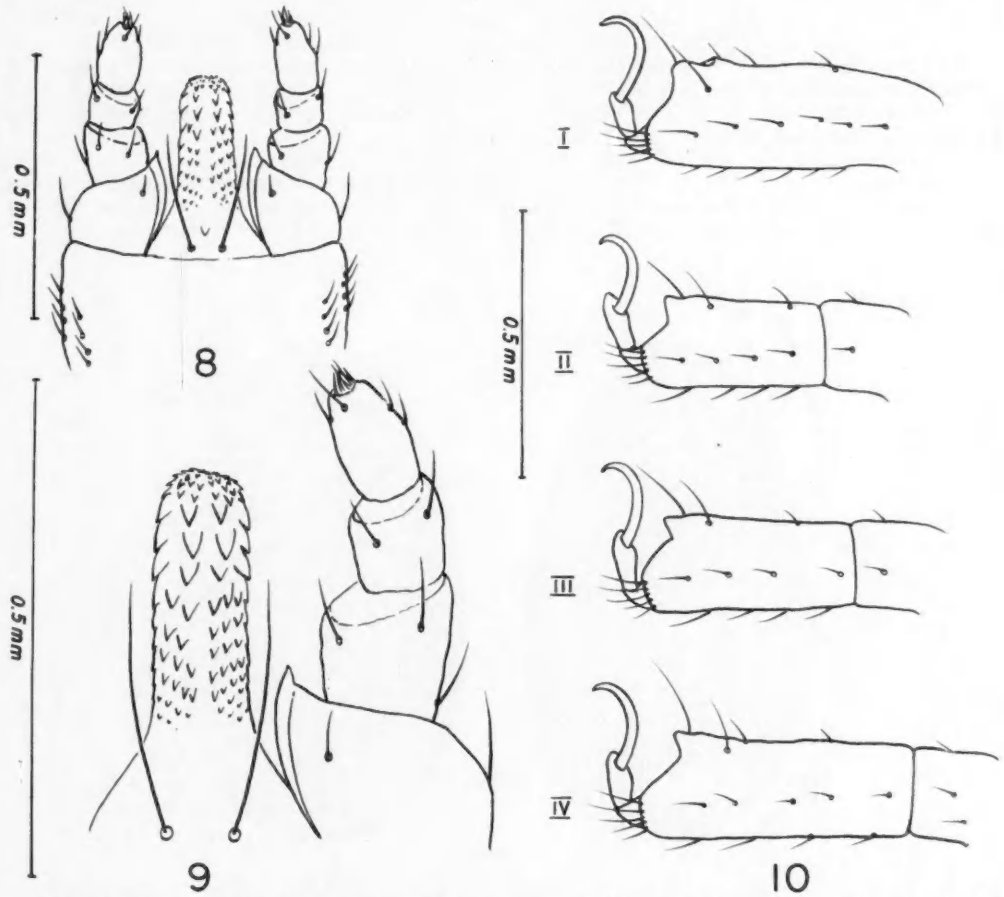
Argas neghmei, n. sp., adult paratypes from Chuquicamata and Calama, Chile. FIG. 5.—Capitulum of female, ventral view. FIG. 6.—Hypostome and palpus of female, enlarged. FIG. 7.—Tarsi I–IV of female, lateral view.

capituli with approximately 10 pairs of lateral and sublateral setae on ventral surface; a pair of post-hypostomal bristles arising slightly anterior of or on level of insertion of palpi and extending approximately to midlength of palpal segment *2*; postpalpal bristles absent. *Hypostome* approximately 2.6 times as long (from level of basal denticles to apex) as wide; lateral margins parallel, apical margin bluntly rounded and bearing a moderate corona; dentition on anterior third of shaft *2/2* with three or four large denticles in the outer file and two or three in the inner file; on basal two-thirds of shaft denticles are much smaller and *3/3* to *5/5* with seven to nine in each file. *Palpi* extending beyond hypostome by length of segments 3 and 4; segment 1 globose, others narrower; segments 1 and *2* subequal in length; segments 3 and 4 subequal, each about two-thirds as long as 1, three or four setae arising from the ventral surface of each segment.

Eyes absent. *Spiracular plates* small, situated laterad of coxa IV. *Genital aperture* forming a transverse slit at level of base of coxae I. *Anus* elliptical, situated slightly posterior to midlength of body, several fine setae arising from each valve. Ventral *"paired organ"* absent. *Coxal* and *supercoxal folds* present.

Legs moderately long and stout; arising from anterior half of body. Coxae II to IV contiguous, I and II separated by narrow integumental area. *Tarsi* (fig. *7*) each with a more or less elevated subapical dorsal protuberance; apical margin distinctly retrograde forming a more or less elevated, triangular process dorsally and a thumblike process ventrally. Tarsal setae visible from lateral view on I to IV typically as follows: apicodorsal *2, 2, 2, 2*; subdorsal 3, 1, 1, 1; median 5, 3, 3, 3; apicoventral 6, 6, 6, 6; subventral 5, 5, 5, 5. *Claws* large, moderately curved apically; *pads* (pulvilli) obsolete.

MALE (figs. 8-12).—Similar to female except for slight secondary sexual characteristics. Measures



Argas neghmei, n. sp., adult paratypes from Chuquicamata and Calama, Chile. FIG. 8.—Capitulum of male, ventral view. FIG. 9.—Hypostome and palpus of male, enlarged. FIG. 10.—Tarsi I-IV of male, lateral view.

from 5.50 mm. to 8.71 mm. in length and from 3.48 mm. to 4.51 mm. in width. The allotype, an average specimen, is 7.38 mm. long and 4.51 mm. wide. *Genital aperture* situated between coxae II; apron forming half a circle. *Capitulum* similar to that of female but arrangement of setae on ventral surface of basis capituli slightly different, the large anterior denticles of hypostome number four or five in the outer row instead of three or four, the basal hypostomal denticles are somewhat fewer and larger, and the setae on palpal segments 1 to 3 appear to be one less than in female. *Tarsal* setal counts, from I to IV, typically as follows: apicodorsal 2, 2, 2, 2; subdorsal 3, 2, 2, 2; median 6, 4, 4, 5; apicoventral 5, 5, 5, 5; subventral 6, 4, 4, 4,

NYMPH.—Similar to adult except for generally smaller size, though large nymphs may equal or exceed some adults in size. No known first-instar nymphs available.

LARVA.—(unengorged, reared, figs. 13 to 17). Body subcircular, measuring approximately 0.72 mm. in diameter (excluding capitulum). An elongate ovate squamous area present on dorsal integument.

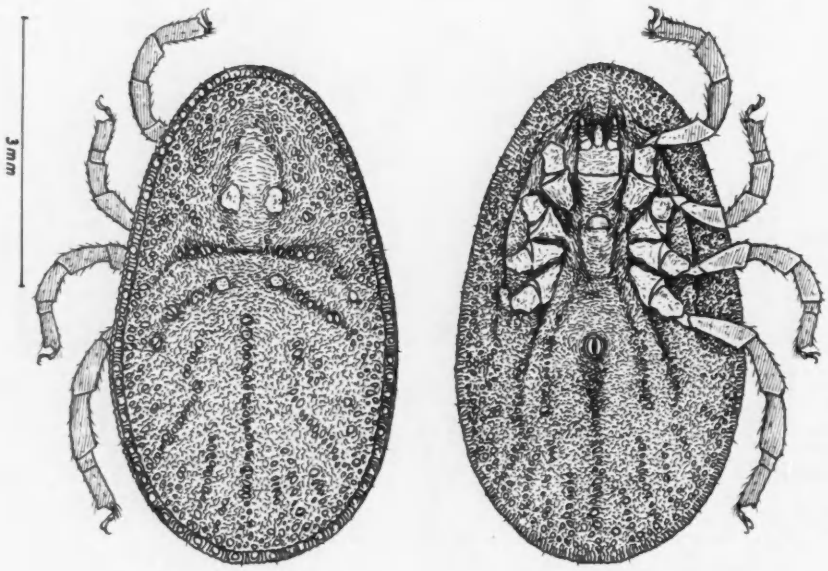
Setae of body and legs fringed on apical half. Body with 20 pairs of dorsolateral setae, plus 2 pairs bordering squamous area, and six setae on each posterior quadrant. Ventral setae of body numbering three pairs in intercoxal area, two setae on each coxa, one seta from each anal valve, and three circumanal pairs.

Capitulum (figs. 15-16).—*Basis capituli* as illustrated, with two pairs of short bristles on ventral surface. *Palpi* with a single strong ventral seta on segments 2 and 3 and three shorter, strong setae ventrally on segment 4 in addition to the apical tuft; segments 1 to 3 subequal in length, segment 4 approximately 1.5 times as long as 3. *Hypostome* three times as long as wide, lateral margins parallel, apical margin bluntly rounded with a small corona; dentition 2/2, denticles large, in files of 9 or 10 extending from corona to base; basal denticles only slightly smaller than apical denticles.

Legs long and stout; *pads* (pulvilli) obsolete; *claws* fairly long; setae as illustrated.

HOLOTYPE.—Female, from a collection taken in dovecotes, chicken houses and nearby human dwellings, Chuquicamata, Province of Antofagasta, northern Chile, February 1949, Dr. Amador Neghme. Deposited in the Rocky Mountain Laboratory collection (RML 27435). ALLOTYPE.—Male, data as for holotype. Deposited in Rocky Mountain Laboratory collection. PARATYPES.—12 ♂, 4 ♀, 11 nymphs, data as for holotype; also 3 larvae reared from this lot. 4 ♂, 1 nymph, with source, date, and collector as above, Calama (20 miles from Chuquicamata), Province of Antofagasta, Chile (RML 27436). 35 ♂, 25 ♀, 150 nymphs from chicken house, Calama, Chile, December 1960, Dr. Hernán Reyes (RML 36957); also numerous larvae reared in the laboratory from this lot.

Paratypes are deposited in the collections of the Rocky Mountain Laboratory, United States National Museum, Department of Parasitology of the University of Chile, and Harry Hoogstraal.



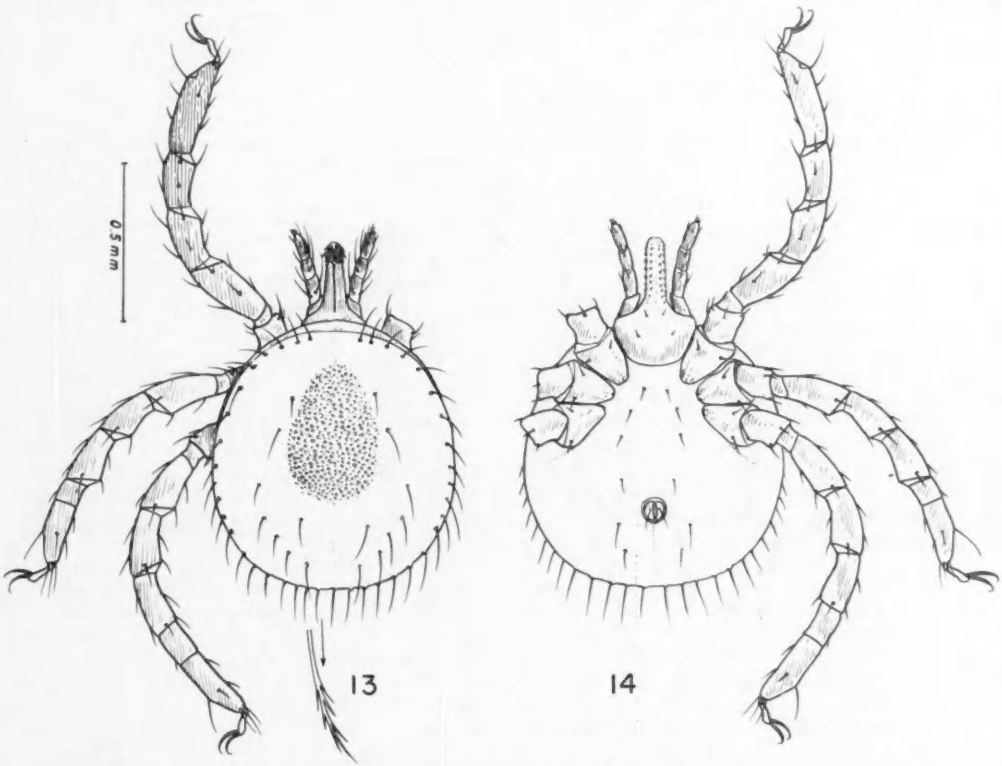
Argas neghmei, n. sp., adult male paratype from Chuquicamata, Chile. FIG. 11.—Dorsal view. FIG. 12.—Ventral view.

DISTRIBUTION.—Although we have seen specimens from only the localities indicated, Dr. Neghme informs us (personal correspondence) that he also found this tick in the very old village of Chiuchiu and in houses scattered along the road from Calama to Chiuchiu. The elevations at which it has been found range from about 7,400 feet at Calama to about 8,800 feet at Chuquicamata.

RELATED SPECIES.—Adults of *A. neghmei* differ from all other species of the *reflexus* group except *A. cucumerinus* Neumann, 1901, in having the dorsolateral integument composed of *persicus*-like "cells" interspersed with areas of *reflexus*-like striations. *A. cucumerinus* is known with certainty only from the original two males found under rocks in dry places at Lima, Peru, by Gaudichaud (Paris Museum). Through the courtesy of Dr. Marc André the senior author has had the privilege of examining a specimen labelled "*Argas cucumerinus* Neumann ♂, auct. det. 1906, Lima, Peru, Gaudichaud 2510-33." Whether this specimen, which agrees well with Neumann's description, can be considered a cotype in view of the 1906 date, is problematical. However, comparison of this specimen and the original description with the Chilean species revealed that *cucumerinus* is about as broad anteriorly as posteriorly, the "cells" of the dorsolateral integument are fewer and less clearly defined, and the peripheral sutural line separating the dorsal and ventral surfaces is less distinct and apparently absent from the posterior one-fourth of the body margin. The single male from Iquitos, Peru, host unknown, which Schulze (1941) believed to be *cucumerinus*, is probably not that species. Except for the statement that the sharp suture between the dorsal and ventral surfaces is lacking, his description and figures suggest that the specimen concerned may be *neghmei*. However, its identity can be determined with certainty only when additional materials, especially larvae, are available for study.

Since *A. neghmei* has been misidentified in the past as *A. persicus*, which occurs widely in South America as well as in many other parts of the world, it may be noted that adults and nymphs of the two are readily distinguished by the presence of long postpalpal hairs in *persicus* as well as by marked differences in the nature of the dorsolateral integument of the two species.

The larvae of *A. neghmei* may be distinguished at once from those of *A. persicus* by their elongate-ovate squamous area (subcircular in *persicus*), the

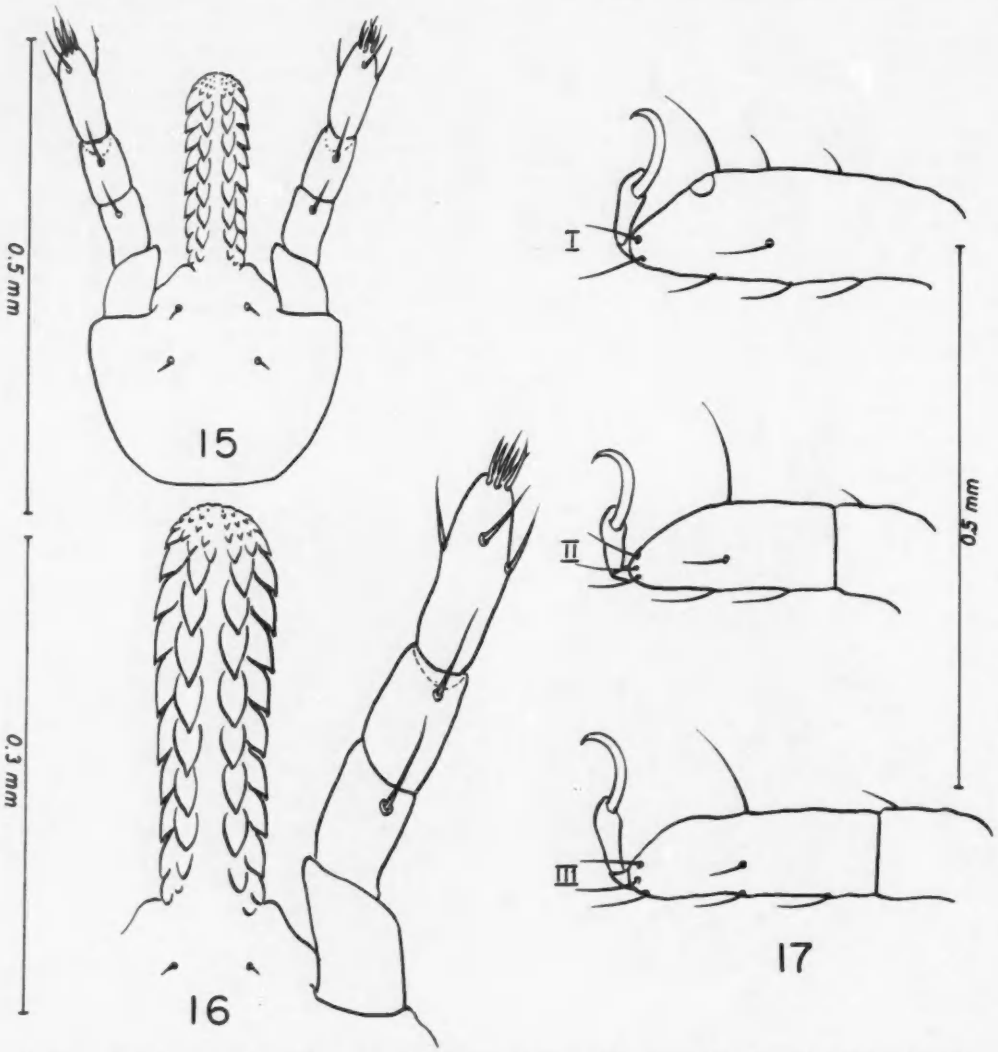


Argas neghmei, n. sp., paratype larva, unengorged, reared from adults from Chuquicamata, Chile. FIG. 13.—Dorsal view. FIG. 14.—Ventral view.

presence of 20 instead of 15 pairs of dorsolateral setae, the presence of 2 pairs instead of 3 pairs of setae bordering the squamous area, and the absence of the anterior submedian dorsal setae situated in *persicus* between the squamous area and the row of setae bordering the anterior body margin.

The larvae of *A. r. reflexus* of Europe resemble those of *A. neghmei* rather closely but have a narrowly rectangular squamous area and many more, and more heavily fringed, dorsal setae (Hoogstraal and Kohls 1960a). The larvae of *A. reflexus hermanni* Audouin of Africa have the hypostomal dentition 3/3, a subcircular squamous area, and fewer dorsal setae (Hoogstraal and Kohls 1960b). Larvae of *A. cooleyi* Kohls and Hoogstraal (1960) of western North America have a more nearly circular squamous area and a shorter hypostome with larger and fewer denticles. Larvae of *A. brevipes* Banks, 1908, of Arizona and California, U.S.A., and Baja California, Mexico (Kohls, Hoogstraal and Clifford 1961) are quite similar to those of *A. neghmei* but have 15 pairs instead of 20 pairs of dorsolateral setae. Additional features for distinguishing larvae of these and other species of *Argas* are given by Sonenshine, Clifford and Kohls (in press).

The only *Argas* larva other than *persicus* and



Argas neghmei, n. sp., paratype larva, unengorged, reared from adults from Chuquicamata, Chile. FIG. 15.—Capitulum, ventral view. FIG. 16.—Hypostome and palpus enlarged. FIG. 17.—Tarsi I-III, lateral view.

neghmei reported from South America is *A. persicus porteri* Lahille, 1915, described from a single larva found on pasture grass on a farm in San Bernardo, Chile. The description and figures leave some doubt as to whether the tick is actually an *Argas*, but they nevertheless indicate that it differs from both *persicus* and *neghmei* in having much heavier legs, in the relative length of the palpal segments, and in having the hypostome shorter and with fewer denticles.

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A Taxonomic Study of the Genus *Cremnops* Foerster in America North of Mexico (Hymenoptera, Braconidae)[1]

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ABSTRACT

The 14 species of *Cremnops* now known from the United States and Canada are redefined and a key is provided for their identification. The males of several species are described for the first time, and the previously known males are redescribed. **C. shenefelti,** from Nevada and California, is a new species; *C. nigrosternum* (Morr.) [(=*C. szepligetii* Morr.)] and *C. californicus* (Morr.) [(=*C. aionos* (Shenefelt)], new synonymy. Also included is a key to the 8 North American genera of the subfamily Agathidinae.

The genus *Cremnops* Foerster was monographed for Nearctic America by H. Morrison in 1917. Morrison separated the species primarily on color, which is quite variable within this genus and, therefore, unreliable. Hence it has been difficult for later investigators to identify accurately many of the species in the genus. It is the purpose of this study to place the species of *Cremnops* occurring in America north of Mexico on a sounder taxonomic basis, and to provide a reasonably accurate key to their identification. Several previously unused taxonomic characters are shown to be of diagnostic value. These are the shape of the head and the mouthparts, particularly the maxillae and mandibles. The male genitalia were studied but found to be quite similar. Two species have been synonymized and one is described as new. In most cases the male is redescribed and in a number of cases is described for the first time. Included is a key to the North American genera of the subfamily Agathidinae. Of great value was the opportunity in 1960 to study the types of Morrison, Muesebeck, and Shenefelt at the United States National Museum, and those of Cresson at the Academy of Natural Sciences, Philadelphia.

ACKNOWLEDGMENTS

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Specimens have been borrowed for this study from the following institutions: Academy of Natural Sciences at Philadelphia, American Museum of Natural History, California Academy of Sciences, Canadian Department of Agriculture, California Insect Survey, Carnegie Museum, Florida State Plant Board, Illinois Natural History Survey, Museum of Comparative Zoology at Harvard, Nevada State Department of Agriculture, Oregon State College, United States National Museum, University of Ari-

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zona, University of California at Davis, University of Kansas, University of Michigan, University of Minnesota, University of Wisconsin.

FAMILY BRACONIDAE

SUBFAMILY AGATHIDINAE

The following combination of characters can be used to distinguish members of the Agathidinae; head transverse, sometimes lengthened or rostriform; clypeus not semicircularly emarginate at apex; maxillary palpi long and conspicuous; occiput immargined; head excavated behind; notaulices usually impressed, occasionally absent; first submarginal and first discoidal cells of forewing confluent (except in *Earinus*), marginal cell very narrow, always complete, radial vein strong and distinct to wing margin, second submarginal cell triangular, subtriangular or quadrate, very rarely absent; abdomen sessile; ovipositor of female varying from barely exserted to longer than abdomen.

KEY TO THE GENERA OF AGATHIDINAE IN NORTH AMERICA

1. Second submarginal cell of forewing quadrate or subquadrate; second abscissa of radial vein longer than first; if not, distal segment of hind trochanter cariniform on lower outer edge 2
   Second submarginal cell triangular; second abscissa of radial vein absent or very indistinct, rarely as long as or longer than first; trochanter never cariniform 5
2. First submarginal cell of forewing completely separated from first discoidal cell; anterior tarsal claws with a large basal tooth; notaulices wanting ..... ***Earinus*** Wesmael
   First submarginal cell confluent with first discoidal cell; anterior tarsal claws cleft; notaulices present 3
3. Face not elongate, malar space very short; distal segment of hind trochanter distinctly cariniform on lower outer edge; ovipositor barely exserted, sheaths shorter than first abdominal segment ..... ***Zelomorpha*** Ashmead
   Face elongate, malar space at least longer than two-thirds the eye height; hind trochanter usually not cariniform; ovipositor strongly exserted, sheaths nearly as long as abdomen 4
4. Frontal impressions margined laterally to ocelli; first abdominal tergum wider at apex than at base; hind femur thick, about four times as long as broad ..... ***Cremnops*** Foerster
   Frontal impressions immargined laterally; first abdominal tergum very narrow, its sides parallel; hind femur slender, about seven times as long as broad ..... ***Megagathis*** Kriechbaumer
5. Maxillary palpi modified to form a long, hollow, five-segmented beak that is nearly as long as head and thorax combined ..... ***Aenigmostomus*** Ashmead
   Maxillary palpi normal 6
6. Tarsal claws simple; abdomen polished ..... ***Crassomicrodus*** Ashmead
   Tarsal claws with a distinct basal tooth; if rarely without tooth, then abdomen sculptured at base 7
7. Apical segment of hind tarsus as long as second segment; posterior basitarsus thickened, somewhat flattened laterally; tarsal claws large; basal two-thirds of forewing hyaline, apical one-third dusky ..... ***Agathirsia*** Westwood
   Apical segment of hind tarsus shorter than second segment; posterior basitarsus slender; tarsal claws somewhat smaller; wings uniformly dusky, occasionally hyaline ..... ***Agathis*** Latreille

Genus *Cremnops* Foerster

Cremnops Foerster, 1862, Verh. Naturhist. Ver. Rheinlande 19: 246. Type: *Ichneumon desertor* L. (=*Agathis deflagrator* Nees). Monobasic.
Bracon of authors, not Fabricius. See Opinion 162, Internatl. Comm. Zool. Nomenclature, 1945.

Fabricius, in his Systema Piezatorum (1804), described the genus *Bracon* with several included species. Curtis (1825) designated *Ichneumon desertor* Linneaus, which Fabricius included in *Bracon*, as the generotype of *Bracon*. The genus *Cremnops* was described by Foerster in 1862 in the subfamily Agathidinae and included a single species, *Agathis deflagrator* Nees. Viereck (1914) showed that *Agathis deflagrator* was the same as *Ichneumon desertor* and, therefore, that *Cremnops* Foerster was isogenotypic with *Bracon* Fabricius and must be suppressed as a synonym. Consequently it became necessary to apply the generic name *Bracon* to those species in the genus *Cremnops*. Furthermore it was necessary to replace the name Agathidinae with the name Braconinae since, under the rules of nomenclature, one of the subfamily names must be based on the same generic name upon which the family name is based. This change was published by Gahan in 1917. In 1945 the International Commission on Zoological Nomenclature, under Opinion 162, suspended the rules in the case of *Cremnops* and designated *Ichneumon minutator* Fabricius as the type of *Bracon*, thus establishing the present-day concept of the genus *Cremnops*.

DESCRIPTION.—Length, 5.5-12 mm.; colors in various shades of red to yellow, usually marked with black; head strongly produced below the eyes, narrowed toward labrum; malar space varying from a little shorter to a little longer than the eye height; ocelli arranged in a nearly equilateral triangle; frontal impressions deep, margined basally and laterally to lateral ocelli, this margin forming two projections between antennae (interantennal plates, fig. 20, ip); antennae 35- to 45-segmented; anterior tentorial pits deeply impressed; labrum usually wider than long, occasionally as wide as long; mandibles bidentate, apical tooth larger than subapical one; maxillae and labium united and elongate, maxillary and labial palpi elongate (fig. 11); apex of pronotum with large, deep pits on each side of midline, these united in one species; notaulices well defined, rarely indistinct, often foveolate; posterior angles of scutum produced into vertically compressed plates, curving inward in one species; scutum with a deep transverse groove in front of scutellum (prescutellar groove); mesopleural furrow present, arising at base of middle coxa and extending forward on mesopleuron, usually foveolate, varying in size and shape; propodeum with three distinct longitudinal carinae on each side and many transverse carinae; wings fuliginous, second submarginal cell of forewing quadrate; legs long, slender except hind femora which

are often short and robust, hind tibia with two or more stout spines at outer distal end; front and middle tarsal claws always cleft, posterior ones usually cleft, but occasionally with a broad basal tooth; abdomen elongate; ovipositor of female usually as long as abdomen, barely exserted in one species; male genitalia with volsellae lying in a horizontal plane between parameres, digiti strongly developed, turning laterally and dorsally on ends of volsellae, aedeagus simple, broad and grooved (description of male genitalia from Snodgrass 1941).

KEY TO THE SPECIES OF THE GENUS *CREMNOPS* IN AMERICA NORTH OF MEXICO

1. Hind tibia with a cluster of four or more stout spines at outer distal end, if apparently with less than four, then malar space distinctly longer than eye height 2
   Hind tibia with two or three stout spines at outer distal end, if rarely with numerous smaller spines above, then malar space shorter than or nearly equal to eye height 6
2. Scape produced into a short tooth on front distal margin (fig. 15) 3
   Scape normal, rounded off in front (fig. 14) 4
3. Hind femur short, robust, about 2½ times as long as broad; ovipositor of female never longer than posterior basitarsus; head and mesopleuron black, remainder of body red ..... ***crassifemur*** (Muesebeck)
   Hind femur longer, slender, about four times as long as broad; ovipositor longer than posterior basitarsus; head and mesopleuron varying from black to yellowish-orange, remainder of body yellowish-orange ..... ***vulgaris*** (Cresson)
4. Mesopleural furrow long, curving upwards, distinctly foveolate ..... ***californicus*** (Morrison)
   Mesopleural furrow short, straight 5
5. Malar space shorter than or nearly equal to eye height; hind femur short and robust, its width nearly equal to half its length ..... ***shenefelti,*** new species
   Malar space distinctly longer than eye height; hind femur slender, normal ..... ***nigrosternum*** (Morrison)
6. Apical tooth of mandible large, extending beyond inner margin of body of mandible (fig. 12); width of lowest portion of face nearly equal to length of malar space (fig. 21); lateral clypeal sutures distinct 7
   Apical tooth of mandible small, not extending beyond inner margin of body of mandible (fig. 13); width of lowest portion of face equal to half the length of malar space (fig. 22); lateral clypeal sutures indistinct 8
7. Length, 7-8 mm.; lowest portion of face as wide as least interocular distance; interantennal plates greatly produced, the groove between them deep and extending down face; notaulices usually weak or indistinct; venter and usually tegula black ..... ***washingtonensis*** (Shenefelt)
   Length, 6-7 mm.; lowest portion of face slightly narrower; interantennal plates weaker, the groove between them shallower; notaulices well defined, groovelike; venter and tegula usually yellow ..... ***melanoptera*** Ashmead
8. Anterior portion of pronotum raised into a prominent erect lamina behind head; apical pits of pronotum fused (figs. 18, 19) ..... ***slossonae*** (Morrison)
   Pronotum normal; apical pits separated 9
9. Posterior angles of scutum produced into finger-like projections on each side of scutellum (fig. 17) ..... ***comstocki*** (Morrison)
   Posterior angles of scutum normal, at most produced into vertically compressed lobes (fig. 16) 10
10. Mesopleural furrow deep, strongly foveolate 11
    Mesopleural furrow shallow (at least anteriorly), weakly or not at all foveolate 13
11. Antennae 35-segmented or less; posterior angles of scutum very prominent, nearly triangular; head and thorax black; length, 6 mm. ..... ***ashmeadi*** (Morrison)
    Antennae more than 35-segmented; posterior angles of scutum somewhat less prominent; length, 7-8 mm. 12
12. Head, prothorax and mesothorax black; scutellum truncate with a definite carinate margin at apex; posterior angles of scutum normally produced ..... ***montrealensis*** (Morrison)
    Head and thorax rusty-orange; scutellum usually rounded off at apex; posterior angles of scutum very slightly produced ..... ***haematodes*** (Brullé)
13. Distal segment of hind trochanter weakly cariniform on lower outer edge; head nearly as broad as high; mesopleural furrow with one or two deep foveae at base, remainder represented by a long shallow non-foveolate groove; entire body yellow ..... ***virginiensis*** (Morrison)
    Hind trochanter not cariniform; head relatively narrow; mesopleural furrow shallow, uniformly but weakly foveolate; head and much of thorax and legs black, rest of body orange ..... ***kelloggii*** (Morrison)

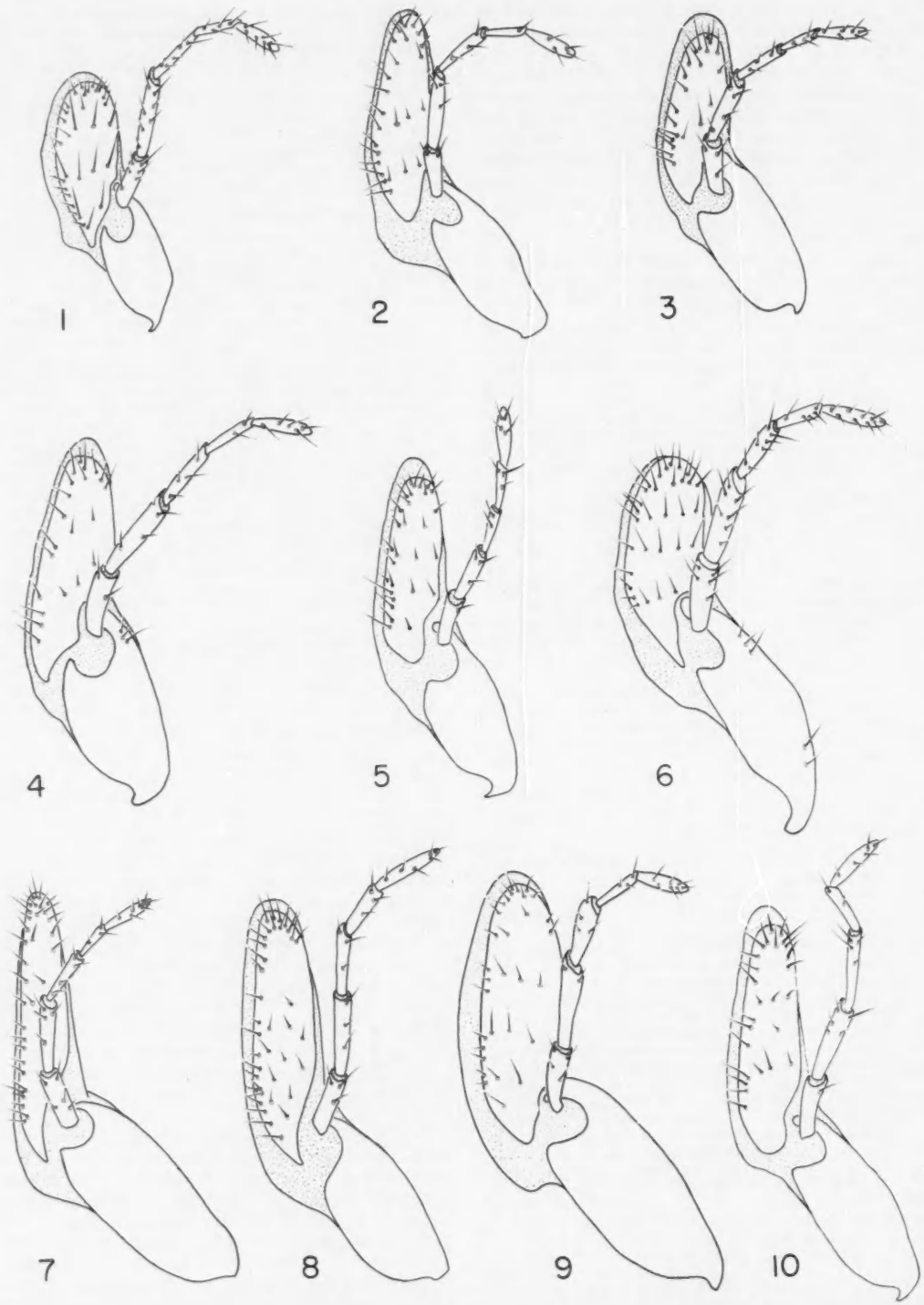
Cremnops crassifemur (Muesebeck)

Bracon crassifemur Muesebeck, 1927, Proc. U. S. Natl. Mus. 69(2642): 9-10. Holotype male from Baboquivari Mountains, Arizona; Univ. of Illinois.

MALE: Length, 9-10 mm.; color red except head, median mesonotal lobe, lower part of propleuron, mesopleuron, venter, tegula, front and middle legs, hind trochanters and tarsi which are black; malar space slightly longer than eye height; scape with a strongly developed tooth on front distal margin (fig. 15); antennae 41- to 43-segmented; labrum wider than long, sides parallel; apical tooth of mandible small, not extending beyond inner margin of body of mandible; galea of maxilla broad, about three times as long as broad (fig. 9); notaulices weakly impressed, nonfoveolate; mesopleural furrow short, composed of a single deep fovea, occasionally with a short depression extending forward; dorsal face of propodeum nearly horizontal in profile, hind face sloping sharply; hind femur very stout and broad; hind tibia stout, with an unusually large number of stout spines at outer distal end; tarsal claws large.

FEMALE: Essentially as in male except antennae 43- to 45-segmented; ovipositor barely exserted, sheaths black.

SYSTEMATICS.—This species appears to be closely related to *vulgaris* by the shape of the scape. It can be separated easily from *vulgaris* and from other *Cremnops* species by its color, the robust femur and body in general, the very short ovipositor in the female, and the very short mesopleural furrow. The only variation in the 17 specimens examined lies in the mesopleural furrow which is composed of a single deep fovea but which may also include a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shallow indistinct depression extending forward.

DISTRIBUTION.—Previous records of this species include Arizona and Texas (Muesebeck 1951). I have examined nine males and eight females, including specimens from southern California as well as Arizona and Texas.

Cremnops vulgaris (Cresson)

Agathis vulgaris Cresson, 1865, Proc. Ent. Soc. Philadelphia 4: 295. Holotype female from Colorado, in Academy of Natural Sciences, Philadelphia.
Agathis media Cresson, 1865, Proc. Ent. Soc. Philadelphia 4: 295. Holotype male from Colorado, in Academy of Natural Sciences, Philadelphia.
Agathis exoratus Cresson, 1872, Trans. Amer. Ent. Soc. 4: 182. Holotype female from Texas, in Academy of Natural Sciences, Philadelphia.

MALE: Length, 7-9 mm.; color yellowish-orange except antennae, cheeks and vertex, venter, tegula, dorsal and ventral portions of propodeum, front and middle coxae and trochanters, hind trochanters, and hind tibiae and tarsi which are black; malar space longer than eye height; scape with a strongly developed tooth on front distal margin; antennae 34- to 36-segmented; labrum longer than wide, sides parallel; apical tooth of mandible large, extending beyond inner margin of body of mandible; galea of maxilla long and narrow, about six times as long as broad (fig. 7); notaulices well defined, foveolate; mesopleural furrow straight, deeply foveolate, extending half the mesopleural length; hind femur and tibia slender, normal; hind tibia with a cluster of about 15 spines at outer distal end.

FEMALE: Essentially as in male except head and propodeum with less black coloration; ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—This species and *crassifemur* differ from all other *Cremnops* by the presence of the tooth on the scape. It can be separated from *crassifemur* by the characters mentioned in the description and in the key. The only significant variation observed was that of color. In both sexes the amount of black varied considerably. The male appears to have more black, particularly on the head, than does the female.

HOST.—I have seen specimens that have been reared from larvae of the pyralids *Loxostege sticticalis* (L.) and *L. commixtalis* (Walkr.).

DISTRIBUTION.—*C. vulgaris* has been recorded from Pensylvania to Louisiana, west to Oregon and Arizona, and from Ontario to British Columbia in Canada (Muesebeck 1951 and Krombein 1958). I have examined over 650 specimens from the entire continental United States, as well as Canada and Mexico.

Cremnops californicus (Morrison)

Bracon californicus Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 331-332. Holotype female from "Sou. Calif."; Cat. No. 20481 U.S.N.M.
Bracon aionos Shenefelt, 1937, Canadian Ent. 69: 205-6. Holotype female from Grand Coulee, Wash.; Cat. No. 52168 U.S.N.M. NEW SYNONYMY.

MALE: Length, 8-10 mm.; color yellowish-orange except head, at least lower part of propleuron, entire mesopleuron, metapleuron, scutellum, propodeum and venter which are black, abdomen, trochanters and apex of hind tibia occasionally marked with black (very rarely body entirely yellowish-orange); head broad, face bi-convex in profile; malar space slightly longer than eye height; antennae 40- to 42-segmented; apical tooth of mandible large, extending beyond inner margin of body of mandible; galea of maxilla about four times as long as broad, nearly as long as first four segments of maxillary palpus (fig. 8); mesopleural furrow well defined, deeply foveolate, long, curving upward; posterior angles of scutum not greatly produced; propodeal carinae well defined; hind tibia with a cluster of about 10 spines at outer distal end.

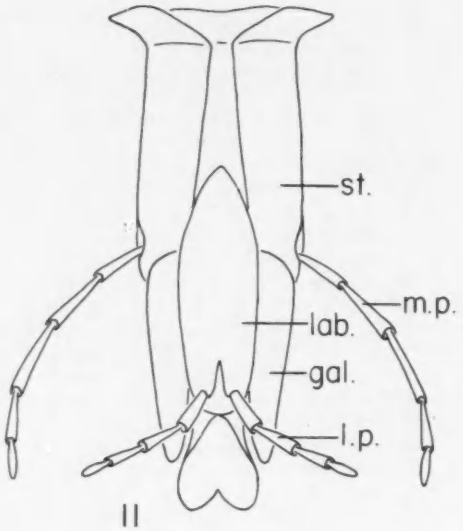
FEMALE: Essentially as in male except those areas indicated as black in the male lighter, or entirely yellowish-orange in the female; ovipositor nearly as long as abdomen, sheaths black.

SYSTEMATICS.—The unusual shape of the mesopleural furrow, with its upward curve, is distinctive for this species. Upon examination of the types of *californicus* and *aionos*, which are both females, it was found that these differed primarily in color and only very slightly in morphology. I have seen large series of *californicus* and *aionos*, each being made up of females exclusively. Included are a number of intermediate forms which cannot be placed accurately into either group. The localities represented within the *californicus* series exactly duplicate those in the *aionos* series, including type localities. I have also seen a large series of male specimens, differing only in color and representing the same localities as the two female series, including type localities again. The only dependable differences between the series of females and the one of males is that of color. Therefore I have concluded that the males are the undescribed males of *californicus* and that *aionos* is a synonym of *californicus*.

The color of the male is generally as in the description except for the following variations: the head, although usually black, is occasionally marked with yellowish-orange on the vertex, the area below the antennae and the clypeus; propleuron is usually yellowish-orange on the upper one-third but is occasionally entirely black. The female varies from entirely yellowish-orange to yellowish-orange except the propodeum, metapleuron and mesopleuron and venter which are black. The head, prothorax, scutum and scutellum are always yellowish-orange.

EXPLANATION OF FIGURES

1. Maxilla, lateral view, *Cremnops comstocki*. 2. Same, *C. shenefelti*. 3. Same, *C. ashmeadi*. 4. Same, *C. virginiensis*. 5. Same, *C. kelloggii*. 6. Same, *C. slossonae*. 7. Same, *C. vulgaris*. 8. Same, *C. californicus*. 9. Same, *C. crassifemur*. 10. Same, *C. nigrosternum*.



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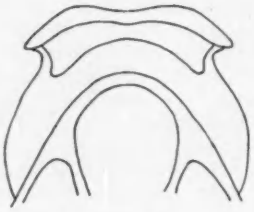

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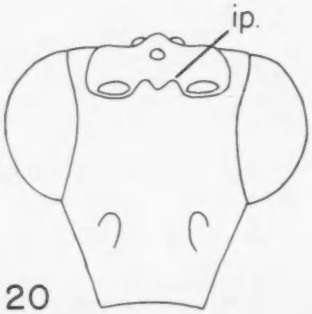

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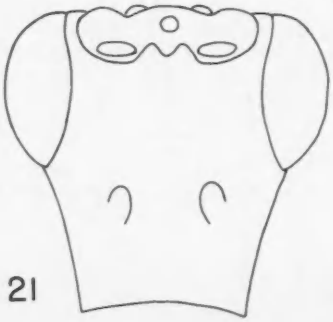

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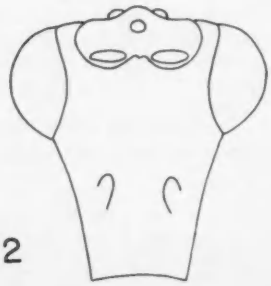

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In both sexes the legs are entirely yellowish-orange except the distal end of the hind tibia and hind tarsus which are black.

I have seen four male specimens, three from Arizona and one from southern California, which are entirely yellowish-orange. Apparently these are structurally the same as *californicus*. The exact placement of these specimens is difficult, but I have included them with *californicus* until more material is available.

DISTRIBUTION.—I have examined 107 females and 65 males from over the entire western United States including the following states: Arizona, California, Colorado, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming. Previous records were only from Arizona, California and Washington (Muesebeck 1951 and Krombein 1958).

Cremnops shenefelti P. M. Marsh, new species

MALE: Length, 7 mm.; color reddish-orange except head, proepisternum, anterior edge of propleuron, lower two-thirds of mesopleuron, tegula, venter, front and middle legs, hind trochanters, tibia, and tarsus which are black; head thick in profile, malar space slightly shorter than eye height; interantennal plates separated by a deep groove; antenna 36-segmented; apical tooth of mandible large, but only slightly extending beyond inner margin of body of mandible; galea of maxilla about 3½ times as long as broad, equal in length to the first three segments of maxillary palpus, these long; second segment twice as long as first (fig. 2); apical pits of pronotum separated; notaulices weakly defined; posterior angles of scutum only slightly produced; mesopleural furrow shallow, wide, weakly foveolate, about one-third as long as mesopleuron; propodeal carinae well developed and uniform; hind femur thick, robust; hind tibia with 13 distal spines; abdomen typical for genus.

FEMALE: Essentially as in male except antennae 37-segmented; ovipositor longer than abdomen, sheaths black.

SYSTEMATICS.—This species is closely related to *kelloggii* but can be separated from it by the greater number of distal spines on the hind tibia, by the thick hind femur and the length of the malar space. Also the propodeum is never black as it sometimes is in *kelloggii* and the propodeal carinae are more distinct. The only color variation appears in the hind tibia which occasionally is invaded by reddish-orange. The number of distal spines on the hind tibia varies from 8 to 15 in both sexes.

Holotype male.—NEVADA: Charleston Mountains, Kyle Canyon, VI-4-41, E. C. Van Dyke. *Paratypes.*—CALIFORNIA: Ivanpah, 8.5 miles S., 1 male, IX-13-55, C. D. McNeill; Panamint Mountains, 1 female, V-29-37, N. W. Frazier; Panamint Springs, 5 miles N.W., 1 female, V-8-58, A. E. Menke; Red Mountain, 1 male, V-11-55, W. R. M. Mason. NEVADA: Fernley, 29 miles N.E., 1 female, V-30-57, T. R. Haig; Charleston Mountains, Kyle Canyon, 2 males, VI-3,4-41, E. C. Van Dyke; Charleston Mountains, Lee Canyon, 1 male, V-25-40, G. E. Bohart.

The type and one paratype have been deposited in the California Academy of Sciences, San Francisco. Other paratypes have been distributed as follows: one in the Canadian Department of Agriculture, Ottawa; two in the collection of the University of California, Davis; two in the United States National Museum; and two in the author's collection. This species is named for Roy D. Shenefelt, professor of entomology at the University of Wisconsin, Madison.

Cremnops nigrosternum (Morrison)

Bracon nigrosternum Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 332-4. Holotype female from Southern Pines, North Carolina; Cat. No. 20479 U.S.N.M.

Bracon szepligetii Morrison, 1917, Proc. U. S. Nat. Mus. 52(2178): 334-6. Holotype female from Calvert, Texas; Cat. No. 20482 U.S.N.M. NEW SYNONYMY.

MALE: Length, 7-10 mm.; color yellowish-orange except at least vertex, middle and hind trochanters, distal one-third of hind tibia and hind tarsus which are black; malar space distinctly longer than eye height (fig. 22); apical tooth of mandible short, not extending beyond inner margin of body of mandible (fig. 13); galea of maxilla about four times as long as broad, nearly equal in length to first three segments of maxillary palpus (fig. 10); antennae 37- to 40-segmented; interantennal plates not very prominent; notaulices well defined, nonfoveolate; posterior angles of scutum usually strongly produced into vertically flattened, almost triangular plates (as viewed from above) on each side of scutellum, but frequently slightly less produced; mesopleural furrow equal in length to one-half the mesopleuron, deep, strongly foveolate; hind tibia with a cluster of 4 to 10 spines at outer distal end, rarely with less than four.

FEMALE: Essentially as in male except those areas indicated as black in male invaded by yellowish-orange in female; ovipositor nearly as long as abdomen, sheaths black.

SYSTEMATICS.—This species can be separated from other *Cremnops* by the length of the malar space and by the number of spines on the hind tibia as well as other characters mentioned in the key and description. The number of spines on the hind tibia varies somewhat, and on one occasion I observed only two present. The color varies from yellowish-orange

EXPLANATION OF FIGURES

11. Mouthparts, posterior view, *C. vulgaris:* gal., galea; lab., labium; l.p., labial palpus; m.p., maxillary palpus; st., stipes. 12. Mandible, *C. washingtonensis.* 13. Mandible, *C. nigrosternum.* 14. Right scape, anterior view, *C. californicus.* 15. Same, *C. crassifemur.* 16. Scutum, dorsal view, *C. kelloggii.* 17. Same, *C. comstocki.* 18. Pronotum, dorsal view, *C. slossonae.* 19. Same, lateral view. 20. Head, anterior view, *C. virginiensis:* ip, interantennal plates. 21. Same, *C. washingtonensis.* 22. Same, *C. nigrosternum.*

with only the middle and hind trochanters, distal one-third of hind tibia and hind tarsus black to having the cheeks, vertex, tegula, venter, fore coxa, trochanters and base of tibia, entire middle leg, hind trochanters, tibia, and tarsus black.

On the basis of type examinations, *nigrosternum* and *szepligetii* appear to be the same species. It is probable that Morrison was observing the extreme opposite ends of a color variable species, as I have seen numerous intermediate forms. The name *nigrosternum* has page priority over *szepligetii*.

DISTRIBUTION.—This species apparently is widespread over the entire continental United States and Canada. Previous records have been from the following states and provinces (Muesebeck 1951 and Krombein 1958): Saskatchewan, Florida, Georgia, Iowa, Louisiana, Massachusetts, Missouri, North Carolina, Pennsylvania, South Dakota, Texas, and Virginia. In addition, I have seen specimens from British Columbia, Arizona, Arkansas, California, Colorado, Kansas, Maryland, Michigan, New Jersey, New Mexico, New York, Utah, and Washington, D. C.

Cremnops melanoptera Ashmead

Cremnops melanoptera Ashmead, 1896, Proc. California Acad. Sci. (2) 4: 124-5. Neotype male from Los Angeles, California; U.S.N.M.

MALE: Length, 6-7 mm.; color yellow except antennae, lower part of proepisternum and venter which are darker; lowest portion of face nearly equal to least interocular distance, entire head nearly as wide as long, malar space slightly less than eye height; interantennal plates usually weakly developed, the groove between them weak; antennae 36- to 37-segmented; apical tooth of mandible long, extending beyond inner margin of body of mandible; galea of maxilla as long as first four segments of maxillary palpus; notaulices distinct, groovelike, nonfoveolate; pronotal pits large, nearly circular; posterior angles of scutum very slightly produced; mesopleural furrow less than half the length of the mesopleuron, deep, distinctly foveolate; propodeal carinae distinct; hind tibia with two or three distal spines.

FEMALE: Essentially as in male except ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—This species falls into the group with fewer than four distal spines on the hind tibia but can be separated easily by the presence of the large apical tooth of the mandible. Also, the width at the apex of the head is greater than in the other species except *washingtonensis*, to which it is most closely related. Occasionally it is difficult to separate *melanoptera* from *washingtonensis*, as both vary in color and in many of the morphological characters mentioned. However, size is always of diagnostic value, as *melanoptera* is smaller than *washingtonensis*. This, along with a combination of the characters mentioned in the key and description, is sufficient to separate the two.

The dark coloration in *melanoptera* varies from light to dark brown. It also varies in amount, particularly on the head and venter. The most significant morphological variation is in the notaulices which are usually well defined and groovelike but occasionally are somewhat weakly defined.

DISTRIBUTION.—I have examined 19 males and 8 females from California, Idaho, Nevada, and Washington. Included is a specimen from eastern Washington labeled as a paratype of *washingtonensis* by Shenefelt but which appears to be *melanoptera*. Previous records for this species were from California (Muesebeck 1951) and Baja California (Ashmead 1896).

Cremnops washingtonensis (Shenefelt)

Bracon washingtonensis Shenefelt, 1937, Canadian Ent. 69: 206-7. Holotype female from Grand Coulee, Washington; Cat. No. 52196 U.S.N.M.

MALE: Length, 7-8 mm.; color yellowish-orange, except at least cheeks, vertex, proepisternum, tegula, venter, foreleg, middle coxa, trochanter, tibia and tarsus, and hind trochanter and tarsus which are black; head slightly longer than wide, breadth of lowest portion of face as great as least interocular distance (fig. 21); malar space about as long as eye height; interantennal plates greatly produced, separated by a deep groove; antennae 37- to 38-segmented; apical tooth of mandible large, extending beyond inner margin of body of mandible (fig. 12); galea of maxilla as long as first four segments of maxillary palpus; notaulices very weakly defined, nearly absent in some cases; pronotal pits narrow, slitlike; posterior angles of scutum very slightly produced; mesopleural furrow straight, short, weak, slightly foveolate and often smooth; propodeal carinae distinct, few transverse carinae present; hind tibia with two or three distal spines.

FEMALE: Essentially as in male except ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—Like *melanoptera*, this species can be separated by the large apical tooth on the mandible, the broad apex of the head, and the number of distal spines on the hind tibia. However, the size of *washingtonensis* is nearly always of diagnostic value in separating it from *melanoptera*.

There is considerable variation in color, particularly of the head which may be entirely black, and the tegula which may be entirely yellowish-orange. The most significant morphological variation is in the notaulices which are usually very indistinct but occasionally are somewhat well defined.

DISTRIBUTION.—Records are from Washington, Colorado, and British Columbia (Muesebeck 1951 and Krombein 1958). In addition I have seen specimens from California, Idaho, Nebraska, Nevada, Oregon and Utah. A total of 37 male and 25 female specimens were examined.

Cremnops slossonae (Morrison)

Bracon slossonae Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 318-20. Holotype female from Biscayne Bay, Florida; Cat. No. 20476 U.S.N.M.

MALE: Length, 10 mm.; color black except metathorax, propodeum, hind coxa, trochanters, femur and middle portion of hind tibia, and abdomen which are red; malar space equal to eye height; head projecting backward to form a strongly arched lobe behind eye; apical tooth of mandible not extending beyond inner margin of body of mandible; galea of maxilla about 2½ times as long as broad, equal in length to first two segments of maxillary palpus (fig. 6); anterior portion of pronotum raised into a prominent erect lamina behind head, apical pits joined forming a transverse groove across pronotum (figs. 18, 19); proepisternum with a thick clothing of fine, silvery hair, mesopleural furrow represented by a short deep impression; propodeum horizontal in profile, hind face sloping sharply; hind tibia with two distal spines.

FEMALE: Essentially as in male except length, 12 mm.; ovipositor as long as abdomen, sheaths shorter and black.

SYSTEMATICS.—This species is very distinct and departs in many ways from the typical *Cremnops*, particularly in the shape of the head, the pronotum, the propodeum, and the mesopleural furrow. From its distribution and habitus it appears to be a tropical species. The specimens examined showed no significant variation.

HOST.—I have seen a male and a female from Florida labeled "Reared from *Epicarsia mellinalis* (Hbn.)."

DISTRIBUTION.—This species has been recorded from Florida (Muesebeck 1951). I have examined seven males and eight females from the following localities in Florida: Biscayne Bay, Buena Vista, Dade County, Key Largo, Matheson Hammock State Park, and Miami.

Cremnops comstocki (Morrison)

Bracon comstocki Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 323-5. Holotype female from Ithaca, New York; Cat. No. 20477 U.S.N.M.

MALE: Length, 7-8 mm.; color black except at least the abdomen which is reddish-orange; malar space about equal to eye height; antennae 40- to 42-segmented; apical tooth of mandible short, not extending beyond inner margin of body of mandible; labrum wider than long, sides not parallel; galea of maxilla about 2½ times as long as broad (fig. 1); posterior angles of scutum produced into inwardly curving, fingerlike projections on each side of scutellum (fig. 17); mesopleural furrow deep, extending forward for about one-half width of mesopleuron; hind tibia long and slender, with two distal spines.

FEMALE: Essentially as in male except antennae 42- to 44-segmented; ovipositor one-half as long as abdomen, sheaths black.

SYSTEMATICS.—*C. comstocki* is easily separated from other *Cremnops* by the unusual shape of the posterior angles of the scutum. There is considerable variation in color within this species. The hind legs vary from entirely black to only the trochanters and tarsi black, the femur and tibia reddish-orange. The metathorax and propodeum vary from entirely reddish-orange to entirely black. Morrison considered these color variations as varieties. However, I have seen considerably more material than Morrison supposedly studied and it appears that there is complete intergradation among the specimens.

DISTRIBUTION.—I have examined 35 male and 19 female specimens from the eastern United States, west to Texas, Kansas, South Dakota and Idaho, and from Saskatchewan, Alberta and Ontario. Localities not previously recorded were from Idaho, Kansas and Nebraska.

Cremnops ashmeadi (Morrison)

Bracon ashmeadi Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 329-31. Holotype female from Robertson, Illinois; Cat. No. 20480 U.S.N.M.

MALE: Length, 6 mm.; color black except abdomen which is reddish-orange; head slightly broader than long, malar space shorter than eye height; apical tooth of mandible not extending beyond inner margin of body of mandible; galea of maxilla about three times as long as broad (fig. 3); antennae 33- to 35-segmented; notaulices well defined, weakly foveolate; posterior angles of scutum produced into rather prominent vertically compressed lobes; median groove of middle lobe of scutum narrow; mesopleural furrow as long as one-half the mesopleuron, deep and strongly foveolate; propodeal carinae indistinct, not uniform; hind tibia with two distal spines; head and thorax covered with long silvery hair.

FEMALE: Essentially as in male except ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—This species is closely related to *montrealensis* and *haematodes* but can be separated easily by its size, color, and the posterior angles of the scutum. Also, there seems to be much more hair on the body of *ashmeadi* than on *montrealensis*. The specimens examined showed no significant variation.

DISTRIBUTION.—This species has been recorded from Illinois, Iowa, Kansas, Kentucky, Minnesota and South Dakota (Muesebeck 1951). I have seen additional specimens from Alberta, Indiana, and Maryland. A total of 14 males and 13 females were examined.

Cremnops montrealensis (Morrison)

Bracon montrealensis Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 326-7. Holotype female from Ithaca, New York; Cat. No. 20483 U.S.N.M.

MALE: Length, 7-8 mm.; color black except at least the abdomen and often the metathorax, propodeum, hind coxa, and hind femur which are reddish-orange; head about as long as broad, malar space shorter than eye height; antennae 37- to 40-segmented; interantennal plates weak, the groove between them weak; apical tooth of mandible small, not extending beyond inner margin of body of mandible; galea of maxilla about three times as long as broad; notaulices distinct, weakly foveolate; median groove of middle lobe of scutum wide; posterior angles of scutum not

greatly produced, a foveolate groove present between the scutum and these angles; mesopleural furrow slightly shorter than one-half the mesopleuron, deep, with three to five large foveae; scutellum broad, truncate, with a definite carinate margin at apex; propodeal carinae distinct; hind tibia with never more than three distal spines.

FEMALE: Essentially as in male except ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—This species, while apparently very close to *haematodes*, can be separated by the scutellum, posterior angles of the scutum, and by the color as mentioned in the description and key. The color of the specimens examined varied somewhat, the parts indicated as reddish-orange occasionally invaded by black. The number of distal spines on the hind tibia varied only from two to three.

HOST.—I have seen a number of specimens which were reared from the pyralid *Evergestis straminalis* Hbn.

DISTRIBUTION.—Records are from the following states and provinces: Quebec, Nova Scotia, Ontario, Michigan to Virginia, and west to Minnesota and Illinois (Muesebeck 1951 and Krombein 1958). I have seen additional specimens which extend the range as far west as Saskatchewan, South Dakota, and Kansas, and as far south as North Carolina, Texas, and Alabama. Total material examined included 66 males and 71 females.

Cremnops haematodes (Brullé)

Agathis haematodes Brullé, 1846, Hist. Nat. Ins. Hym. 4: 495. Holotype male from Philadelphia, Pennsylvania; type location unknown.
Agathis meablis Cresson, 1872, Trans. Amer. Ent. Soc. 4: 183. Holotype male from Texas; Academy of Natural Sciences, Philadelphia.

MALE: Length, 6-8 mm.; color rusty-orange except at least vertex, cheeks, antenna, proepisternum, tegula, front and middle coxae and legs, hind trochanters, apex of hind tibia and tarsus which are black; head as broad as long, malar space shorter than eye height; antennae 37- to 40-segmented; interantennal plates fairly distinct, separated by a shallow groove; labrum as wide as or slightly wider than long; apical tooth of mandible small, not extending beyond inner margin of body of mandible; galea of maxilla about three times as long as broad; notaulices fairly distinct, smooth or weakly foveolate; posterior angles of scutum only slightly produced; mesopleural furrow shorter than one-half the mesopleuron, deep, usually with three to five deep foveae; propodeal carinae distinct; hind tibia with 2 to about 13 distal spines.

FEMALE: Essentially as in male except ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—The color, short malar space, shape of the scutellum, prescutellar groove and posterior angles of the scutum are distinctive for this species. It appears to be the most variable species in the genus. The head ranges from nearly all rusty-orange to entirely black. The venter is occasionally black as are the hind legs. The number of distal spines on the hind tibia is usually two or three but I have seen some variation up to a total of 13. There seems to be no correlation between these variations and geographical distribution or other morphological characters.

HOST.—I have seen specimens that were reared from *Loxostege similalis* (Guen.).

DISTRIBUTION.—This is a widespread species in the United States, previously having been recorded from Pennsylvania to Georgia, west to Colorado and Texas, and from Oregon (Muesebeck 1951). In addition, I have seen specimens from Arizona, California and Florida. A total of 68 males and 76 females were examined.

Cremnops virginiensis (Morrison)

Bracon virginiensis Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 341-3. Holotype female from Falls Church, Virginia; Cat. No. 20484 U.S.N.M.

MALE: Length, 7-8 mm.; color yellow except antennae, apex of hind tibia, and hind tarsus which are black; head distinctly broader than long, thick in profile, malar space shorter than eye height (fig. 20); interantennal plates prominent; labrum as long as wide, sides parallel; galea of maxilla about three times as long as broad, equal to first 2½ segments of maxillary palpus (fig. 4); apical tooth of mandible not extending beyond inner margin of body of mandible; antennae 40- to 42-segmented; notaulices distinct; middle lobe of scutum with a distinct, median, flat groove; posterior angles of scutum only slightly produced; mesopleural furrow short, one or two foveae at base, remainder nonfoveolate and occasionally fading out entirely; distal segment of hind trochanter cariniform on lower outer edge (at least anteriorly); hind tibia with two spines at distal end.

FEMALE: Essentially as in male except ovipositor nearly as long as abdomen, sheaths black.

SYSTEMATICS.—This species is easily separated from other *Cremnops* by the weak mesopleural furrow, broad head, generally large body and the short carina on the posterior segment of the hind trochanter.

DISTRIBUTION.—The recorded distribution of *virginiensis* is from Connecticut to Florida, west to Iowa, Mississippi, South Dakota, and in Quebec and Ontario (Muesebeck 1951 and Krombein 1958). In addition, from among the 74 males and 43 females which I examined were specimens from as far west as Washington, Colorado, Arizona, Texas, and Kansas, and as far north as Maine.

Cremnops kelloggii (Morrison)

Bracon kelloggii Morrison, 1917, Proc. U. S. Natl. Mus. 52(2178): 327-9. Holotype female from Nevada; Cat. No. 20478 U.S.N.M.

MALE: Length, 6-7 mm.; color black except at least the scutum, upper portion of propleuron and mesopleuron, hind femur and abdomen which are orange; head as broad as long, thick in profile, malar space equal to eye height; antennae 31- to 33-

segmented; apical tooth of mandible not extending beyond inner margin of body of mandible; galea of maxilla about three times as long as broad (fig. 5); first segment of maxillary palpus as long as second; notaulices distinct, smooth; mesopleural short, narrow, shallow, uniformly but weakly foveolate; propodeal carinae weak and nonuniform, number of transverse carinae often reduced; hind trochanter not cariniform; breadth of hind femur less than one-half its length; hind tibia with two or three distal spines.

FEMALE: Essentially as in male except ovipositor as long as abdomen, sheaths black.

SYSTEMATICS.—The very weak mesopleural furrow is noteworthy. This species is very close to *shenefelti* but can be easily distinguished by the fewer number of distal spines on the hind tibia, more slender hind femur, and, to a certain extent, by the body markings. There was considerable variation in the color of the propodeum and mesopleuron among the specimens examined. The range was from entirely black to entirely orange.

DISTRIBUTION.—Of the 24 males and 35 females which I examined, all but two were from California. These two, the type and allotype, were labeled "Nev."

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The Skeletomuscular Mechanism of the So-Called Anal Legs in the Centipede *Scolopendra amazonica* (Scolopendridae)[1]

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ABSTRACT

The so-called anal legs of *Scolopendra* belonging to the 21st post-maxillipedal segment are the longest pair of legs and, unlike other legs, they do not participate in locomotion. Correlated with their inability to take part in walking, are found some pecularities in their skeletomuscular mechanism: Their implantation posteriorly on the body articulation with the compound coxopleural plates by an anteriorly situated loose pivot joint, reduction of extrinsic muscles to seven pairs only, and intrinsic muscles disposed mainly for levation and stronger flexion movements between the segments.

The trunk musculature of centipedes has been previously described by several authors (Walton 1903, Bekker 1926, 1949, Verhoeff 1925, Bücherl 1940, Jangi 1961), but these accounts contain hardly any consideration of the musculature of the terminal segments. Several gaps still existing in our knowledge of the breeding phenomena in centipedes call for a careful and thorough investigation of the skeletomuscular mechanisms in their caudal region. The anal legs are particularly of interest. These are larger than other legs and are well-developed in both the sexes. Yet they play no propulsive role during normal locomotion, nor have they been implicated in sexual activities. The present paper describes the skeletomuscular mechanism of these legs in the centipede *Scolopendra*, with the hope that this information will provide a firmer basis for future functional and behavioral studies of these organs. Muscles of the genito-anal complex will be dealt with separately.

MATERIALS AND METHODS

The present study is based on specimens of the centipede *Scolopendra amazonica* Bücherl taken at Nagpur, India. The material was fixed in 10% neutral formalin and the muscles were studied by dissection under the stereoscopic binocular microscope.

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SKELETAL PLAN OF THE 21ST LEG-BEARING SEGMENT

This last leg-bearing segment is covered dorsally by an anteriorly and laterally emarginated tergum bearing a distinct longitudinal and median sulcus (figs. 1, 5 and 15), ventrally by a less extensive, posteriorly tapering and rounded sternum (figs. 2 and 5), and laterally, on each side, by an obliquely placed coxopleuron (fig. 5) which is a heavily sclerotized compound plate formed by the fusion of the coxal and pleural components. The coxopleuron is ventromedially perforated by numerous circular openings of the underlying coxopleural glands. Each of the coxopleural plates (fig. 2) is projected posteromedially on the ventral side into a conical coxopleural process tipped with four spines, and itself bears a posterior thorn (fig. 2). The membranous connection of the coxopleural plates with the tergum and the sternum is scanty and consequently severely limits their mutually relative movements: a fairly compact and stable encasement is thus provided for the organs lying within this segment.

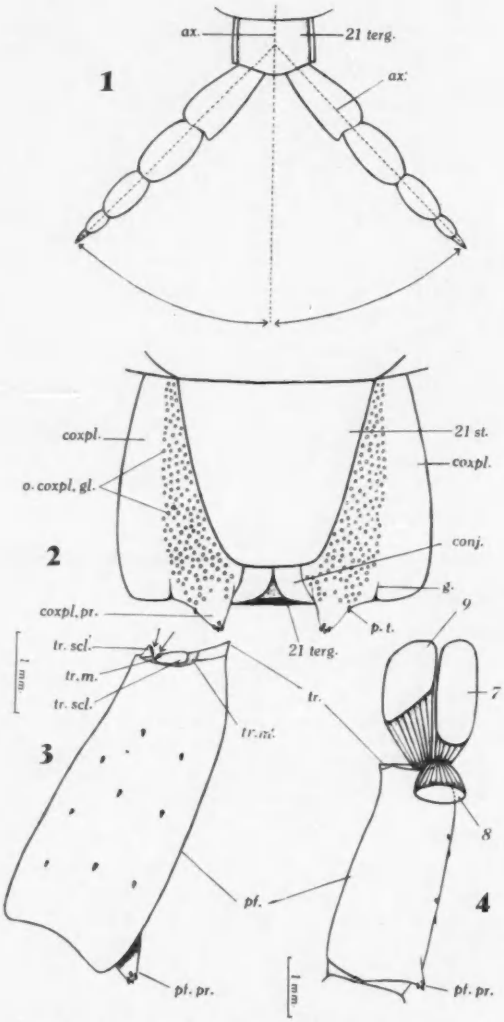


FIG. 1.—Caudal end of *Scolopendra amazonica*, dorsal view. The anal legs are shown in their maximum abduction position.

FIG. 2.—Caudal end (without anal legs), ventral view.

FIG. 3.—Trochanter and prefemur of right leg, detached from body, ventral view; magnification same as in fig. 2. Arrows point to the sclerotized articular points (shown in thick line) on the trochanteral sclerites.

FIG. 4.—Trochanter and prefemur of left leg with the median levators, dorsal view.

MORPHOLOGY OF THE ANAL LEGS

The so-called anal legs do not correspond to the anal segment but are so named on account of their close proximity to the anal region. They are the longest of all the postcephalic appendages and their telopodite, which is the functional appendage, consists of seven segments: first trochanter, second trochanter (prefemur), femur, tibia, first tarsus, second tarsus and pretarsus or claw (figs. 3, 4 and 7).

Unlike the situation in other legs, which are implanted into the body laterally, the anal leg foramina are located posteriorly in the conjunctival membrane which connects the 21st leg-bearing segment with the comparatively much smaller segments of the genito-anal complex (fig. 5). Consequent upon this positional shift, the posteriorly directed anal legs with their axes normally close to and nearly parallel with the body axis have a radius of movement in a forward direction that is limited to 45° (fig. 1). It would, therefore, be more appropriate to refer to their preaxial and postaxial surfaces as *lateral* and *median* instead of *anterior* and *posterior* (as is otherwise done in respect of other legs). The promotion and remotion movements of the anal legs will thus be of abduction and adduction.

The first trochanter, which is relatively much smaller than the second, is firmly united with the latter and does not form a complete ring (figs. 2 and 3). The pivot joint between the leg and the coxopleuron is laterally (anteriorly) situated (fig. 7). Ventrolaterally, between the coxopleuron and the prefemur, occur two trochanteral sclerites of which the more ventrally situated is larger and ovoid and not so movable as the smaller, triangular one (figs. 3 and 6): the mobility of the smaller one is due to more ample arthrodial membrane which connects it with the prefemur (fig. 6). On detaching an anal leg, a short ridgelike apodeme is seen at the posterior margin of the coxopleuron (fig. 5). The external manifestation of this apodeme is a short, longitudinal groove towards the posterior coxopleural border (fig. 2). On the posterior face of this apodeme, two amber-colored breaking points represent the articular points of the aforementioned sclerites (fig. 5). The more superficial and extensive point marks the articulation of the smaller tri-

angular sclerite, while with the deeper one articulates the larger sclerite. It may be noted that while the entire ventral margin of the smaller trochanteral sclerite participates in the articulation, the articulation of the larger one is limited to a much smaller region on its rim dorsally (figs. 3 and 6). Thus, the smaller trochanteral sclerite is mechanically obliged to get deflected outwards so as to stand vertically against the larger. The trochanteral foramen is elliptical in outline and is obliquely placed so that its major axis makes an acute angle with the vertical plane. The elliptical trochanteral foramen serves the functional need of the leg whose movements are predominantly upward and outward, i.e., levation and abduction. The axis of movement—i.e., the line joining the two articular points on the coxopleural apodeme—coincides with the minor axis of the elliptical trochanteral foramen, which, of course makes

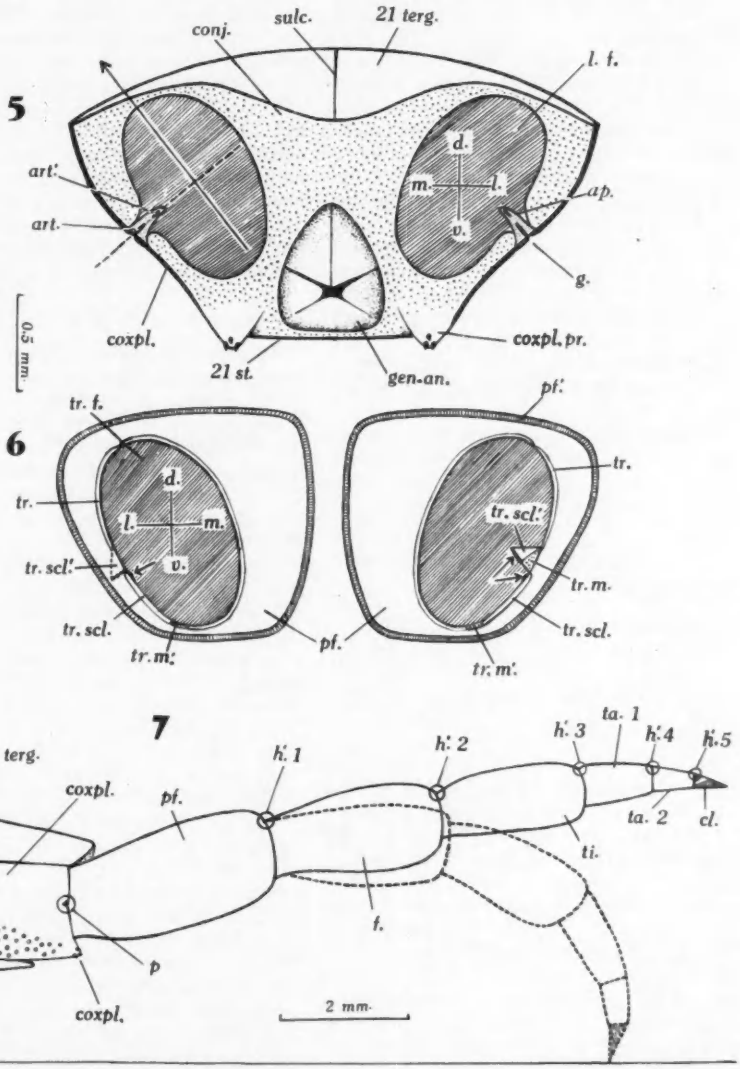


FIG. 5.—Posterior view of caudal end after removal of the anal legs, showing the obliquely directed anal leg foramina and the articular apodemes.

FIG. 6.—Posterior view of the two anal legs in their natural resting position. The legs have been cut transversely near the proximal end. Magnification same as in fig. 5.

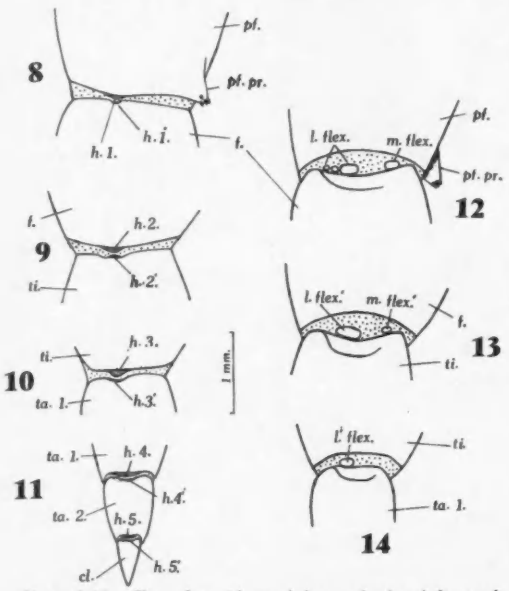
FIG. 7. Left lateral view of caudal region, showing the position of the left anal leg as it is held during locomotion (shown in continuous lines) and in rest (shown in dotted lines). Note that the claw touches the ground during rest.

a right angle with the major axis representing the plane of the major leg-movement (fig. 5).

While the first trochanter and prefemur are almost completely immovably joined to each other, the membranous connection between the successive leg segments permits the bending of one segment over the other. The intersegmental membrane is most extensive ventrally and becomes gradually shortened dorsally till, at the hinge joint, it is insignificant. At a hinge joint, the cuticle is tucked in backwards while the intucking is just in the reverse direction elsewhere in between the two segments (fig. 24). The hinge articulation consists of a backwardly extending and heavily sclerotized articular process from a proximal leg segment: on this process rests (during maximum levation) the sclerotized, knob-like distal component of the hinge. This knob arises by a backward intucking and sclerotization of the cuticle on the localized proximal zone of the leg segment and, like the olecranon process in the forelimb of vertebrates, it does not permit an upward flexion of a more distal segment on the proximal one beyond setting the former in straight line with the latter. The hinge articulations become more and more transversely extended towards the distal part of the leg. The center of each of the hinge articulations is situated slightly on the lateral side of the leg (fig. 8-11 and 25-34).

Proximally the ventral ends of the femur, tibia, and first tarsus are rendered flexible by the presence of a posteriorly curved sulcus and movable femoral, tibial, and tarsal sclerites which may be as many as four, two, and one, respectively.

The anal legs in this species are important inasmuch as they show sexual dimorphism: dorsally in the males, the prefemur, femur, and tibia are flat and have elevated lateral and posterior margins, with a little median interruption on the posterior border of prefemur and femur; while in the females these three segments have dorsally a rounded surface and lack emargination. The prefemur in both sexes carries spines, the number and arrangement of which show some individual variation; ventrolateral spines are generally in three rows of three each (fig. 3), although there may be one more or one less in one or more of the rows, while the dorsomedial spines are generally four in number, arranged medially in two rows, and the median prefemoral process situated distally is tipped with four spines. The first tarsal segment is devoid of the characteristic spur although the claw, as in other legs, carries proximally two ventral spurs.



FIGS. 8-11.—The five hinge joints of the left anal leg in dorsal view. The leg segments have been slightly pulled apart to show the otherwise telescoped articular processes of the prefemur, femur, tibia, first tarsus, and second tarsus.

FIGS. 12-14.—Prefemorofemoral, femorotibial, and tibiotarsal junctions of the right leg, ventral view.

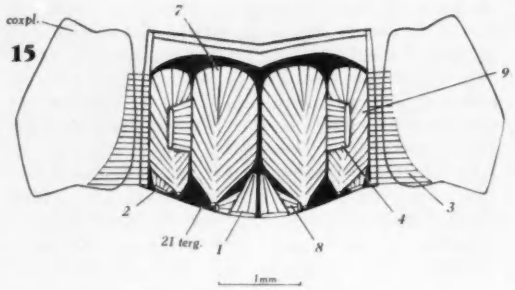
MUSCLES OF THE 21ST LEG-BEARING SEGMENT

NONPEDAL MUSCLES.—The muscles, which are intersegmental in respect of this segment and segments of the genito-anal complex, will be described elsewhere and are, therefore, excluded from the present consideration. Under this head all muscles will be treated which are not inserted on the legs: these muscles, although designated as "nonpedal," do seem to exercise an indirect control on the movement of anal legs.

(a) *Tergoconjunctival Muscles.*—They consist of two paired muscles, viz. *median tergoconjunctival* (1) and *lateral tergoconjunctival* (2)—both originating posteriorly on the tergum (fig. 15). They are inserted on the conjunctiva which connects the 21st segment with the genito-anal region and in which the latter remains wrapped up while telescoped into the body. Apparently these muscles, by virtue of their insertion in close proximity with the leg-base, control to some extent the displacement of the conjunctival membrane during the leg-movement.

(b) *Tergocoxopleural Muscles.*—They form a single pair and each (3) is rather a thin, short, and posteriorly more extensive muscle whose fibres run vertically to connect the lateral border of the tergum with the dorsal region of the coxopleural plate (fig. 15). These muscles control the relative movements of compression between the coxopleura and the tergum, necessary during levation of the legs.

(c) *Tergosternal Muscles.*—They are represented by a single pair of flat muscles (4) gradually narrowing downward (figs. 15 and 16). Each of these muscles originates in a lengthwise manner on the tergum between the median and the lateral levators of the leg and is inserted on an archlike tendon, the median arm of which merges into a longitudinal tendinous intersection of the posterior sternocoxopleural muscle while the vertical arm is implanted on



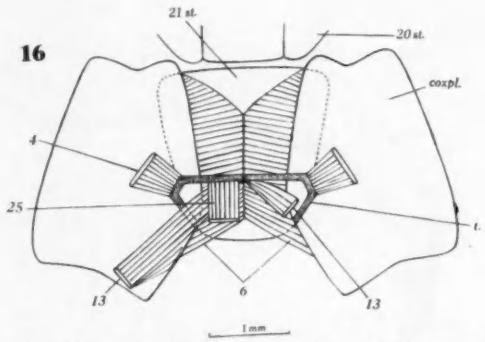
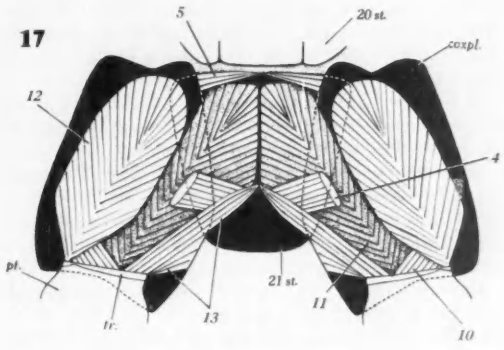


FIG. 15.—Inner view of the muscles originating on the 21st tergum. The coxopleura have been flattened out laterad.

FIG. 16.—Inner view of the posterior sternocoxopleural muscle and the archlike tendon of the tergosternal muscles.



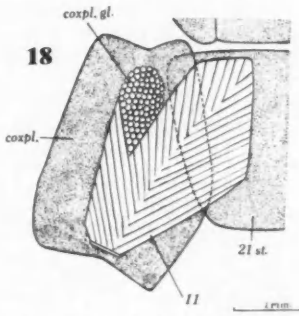


FIG. 17.—Inner view of the extrinsic anal leg muscles. The coxopleura have been flattened out laterad.

FIG. 18.—Inner view of the flexor muscle of the left anal leg. The cluster of coxopleural glands is partly visible after removal of the abductor muscle of the anal leg.

the ventral edge of the coxopleuron very near the posterior border of the sternum. This tendon, with the help of the tergosternal muscle which maintains in it the required tension, offers the necessary leverage for the leg and genital muscles originating on it.

(d) *Sternocoxopleural Muscles.—Anterior sternocoxopleural* (5) is a paired transverse muscle originating on the median line at the anteriormost point on the sternum to be inserted ventrally at the anterior end of the coxopleuron (fig. 17).

Posterior sternocoxopleural (6) is a paired muscle, the fibres of which originate mostly along the median line on a longitudinal tendinous intersection and are inserted on the ventral border of the coxopleura (fig. 16).

These muscles act mainly as adductors of the coxopleura.

PEDAL MUSCLES.—(a) *Extrinsic Muscles.*—They form a system of four levators, a depressor, an adductor, and an abductor on each side.

Anterior median levator (7) is a stout, penniform muscle originating almost along the entire length of the tergum and inserted dorsomedially on the trochanter by a tendon, although some of its posteriormost dorsal fibres are inserted direct on the trochanter (figs. 4 and 5).

Posterior median levator (8) is a small conical muscle originating dorsally to the medial tergoconjunctival muscle (fig. 15). Its converging fibres are inserted dorsomedially direct on the trochanter (fig. 5).

Superior external levator (9) is also a penniform muscle (fig. 15) and is as long as the anterior medial levator although its area of attachment on the tergum is not so extensive (fig. 4). It is inserted dorsally on the trochanter by its tendon in the same manner as the anterior median levator but external to this muscle.

Inferior external levator (10) is a short, flat muscle originating posteriorly on the coxopleural plate, covering of the posterodorsal fibres of the depressor muscle (fig. 17).

Depressor (11) is the longest of the extrinsic pedal muscles. The fibres of this penniform muscle originate both on the sternum and the coxopleural plate and are inserted on a tendon by which it is connected ventrally on the larger trochanteral sclerite. This muscle together with the coxopleural fibres of the depressor cover up the cluster of coxopleural glands which they press upon during contraction (figs. 17 and 18).

Abductor (12) is another pinnate muscle and is diagonally directed. Its fibres originating on the

coxopleuron, join on a tendon by which it inserts dorsolaterally on the trochanter. Besides abduction, which is its major action, this muscle simultaneously helps the levation of the limb (fig. 17).

Adductor (13) is a two-headed muscle. The fibres of its longer bundle originate on the median arm of the arch-shaped tendon while the smaller bundle originates on the coxopleural plate. The fibres are inserted on the tendon of the anteromedian levator as well as direct on the trochanter medially (fig 17).

(b) *Intrinsic muscles.—Median flexor* (14) of the femur is a pinnate muscle, the fibres of which originate dorsally and laterally on the median side of the prefemur and are inserted on the medial sclerite of the femur by way of a tendon: some of its fibres are directly inserted ventromedially on the proximal border of the prefemur. While the fibres attached on the tendon bring about the depressor movement those attached on the femur directly are responsible for the movement of abduction (figs. 19 and 26).

Lateral flexor (15) of the femur (figs. 20 and 26) is a corresponding muscle on the lateral side and is similar in nature except that it is more extensive, stronger, and is inserted by its tendon on the lateral sclerite of the femur: some of the fibres however are attached direct ventrolaterally on the proximal border of the prefemur. On the similar principle operating in the previous muscle, this muscle acts as a depressor as well as an abductor.

Median levator (16) of the femur (figs. 21 and 26) is a pinnate muscle with dorsal and lateral dorsomedially on the femur, which it moves inwards and upwards.

Lateral levator (17) of the femur (figs. 22 and

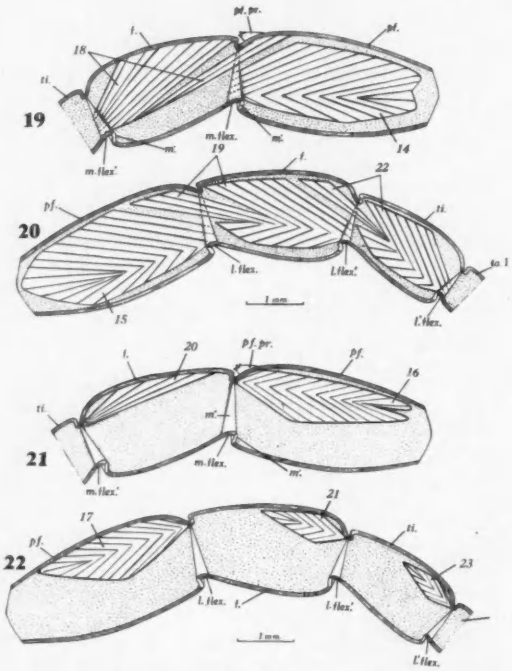


FIG. 19.—Sagittal section of the right anal leg, showing in inner view the femoral and tibial flexors of the medial half.

FIG. 20.—Sagittal section of the right anal leg, showing in inner view the femoral, tibial, and first-tarsal flexors of the lateral half.

FIG. 21.—Sagittal section of the right anal leg, showing the femoral and tibial levators of the medial half.

FIG. 22.—Sagittal section of the right anal leg, showing the lateral levators or the femur, tibia, and first tarsus.

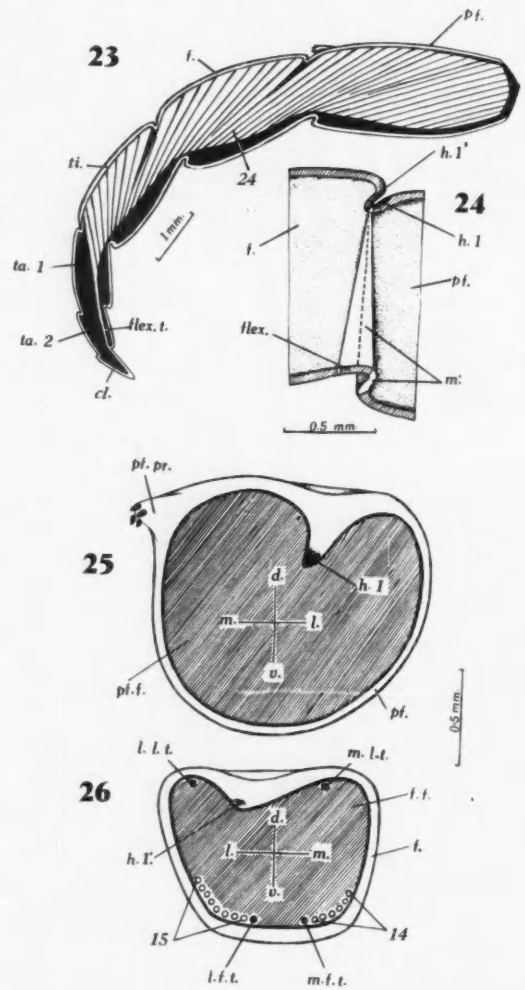


FIG. 23.—Sagittal section of the right anal leg, showing the flexor muscle of the claw.

FIG. 24.—Magnified sketch of the sagittal section of the leg at the level of the first hinge joint.

FIG. 25.—Posterior view of the right prefemur, showing the distal foramen.

FIG. 26.—Anterior view of the right femur, showing the proximal foramen.

26) is a similar muscle found on the lateral side and moves the femur outwards and upwards.

Median flexor (18) of the tibia (figs. 19 and 28) is another pinnate muscle originating dorsally on the medial side of the femur and on the posterior one-third of the prefemur. It is inserted by its tendon on the medial sclerite of the tibia although some of its fibres, as in the median and lateral flexors of femur, are inserted direct on the tibia.

Lateral flexor (19) of the tibia (figs. 20 and 28) is a similar muscle on the lateral side, but its fibres originate laterally and ventrally. Its tendon is inserted on the lateral sclerite of the tibia.
origin external to the median flexor. It is inserted

Median levator (20) of the tibia (figs. 21 and 28) occurs between the median and lateral flexors of this segment and is a pinnate muscle the fibres of which originate dorsally. It is attached by a tendon dorsally on the proximal edge of the tibia externally to the hinge joint.

Lateral levator (21) of the tibia (figs. 22 and 28) is a muscle similar in nature and disposition to the lateral levator of femur but is smaller in size.

Lateral flexor (22) of the first tarsus (figs. 20 and 30) is similar in nature and disposition to the lateral flexor of the tibia.

Lateral levator (23) of the first tarsus (figs. 22 and 30) is a muscle similar in nature and disposition to the lateral levators of femur and tibia but is smaller in size. Such a muscle is absent on the medial side.

Flexor (24) of the claw (fig. 23) is the longest and the stoutest of the intrinsic leg muscles, its fibres originating dorsally on the prefemur, femur, and tibia and are inserted on a long tendon which arises from the pretarsus ventrally.

KEY TO REFERENCE LETTERING

ap.	coxopleural apodeme.
art.	articular point of the larger trochanteral sclerite.
art'.	articular point of the smaller trochanteral sclerite.
ax.	body-axis.
ax'.	leg-axis.
cl.	claw (pretarsus).
cl.f.	claw foramen.
conj.	conjunctival membrane.
coxpl.	coxopleuron.
coxpl. gl.	coxopleural glands.
coxpl. pl.	coxopleural plate.
d.	dorsal.
f.	femur.
f.f.	proximal femoral foramen.
f.f'.	distal femoral foramen.
flex.	flexor sclerite.
flex t.	flexor tendon of the claw.
g.	coxopleural groove.
gen-an.	genito-anal complex.
h.1.	prefemoral component of the first hinge-joint.
h.1'.	femoral component of the first hinge-joint.
h.2.	femoral component of the second hinge-joint.
h.2'.	tibial component of the second hinge-joint.
h.3.	tibial component of the third hinge-joint.
h.3'.	first-tarsal component of the third hinge-joint.
h.4.	first-tarsal component of the fourth hinge-joint.
h.4'.	second-tarsal component of the fourth hinge-joint.
h.5.	second-tarsal component of the fifth hinge-joint.
h.5'	pretarsal component of the fifth hinge-joint.
h.'1-h.'5.	hinge joints.
l.	lateral.
l.f.	leg foramen.
l. flex.	lateral flexor sclerite of femur.
l. flex'.	lateral flexor sclerite of tibia.
l'. flex.	lateral flexor sclerite of first tarsus.
l.f.t.	lateral flexor tendon of femur.
l.f.t'.	lateral flexor tendon of tibia.
l'.f.t.	lateral flexor tendon of first tarsus.
l.l.t.	lateral levator tendon of tibia.
l.l.t'.	lateral levator tendon of first tarsus.
m.	median.
m'.	intersegmental membrane.
m. flex.	median flexor sclerite of femur.
m. flex'.	median flexor sclerite of tibia.
m.f.t.	median flexor tendon of femur.
m.f.t'.	median flexor tendon of tibia.
m.l.t.	median levator tendon of femur.
m.l.t'.	median levator tendon of tibia.
o. coxpl. gl.	openings of coxopleural glands.
p.	coxopleuro-trochanteral pivot joint.
pf.	prefemur.
pf'	cut edge of prefemur.
pf.pr.	prefemoral process.
p.t.	posterior thorn.
sulc.	sulcus.
ta.1.	first tarsus.
ta.2.	second tarsus.
ta.1.f.	proximal foramen of first tarsus.
ta.1.f'.	distal foramen of first tarsus.
ta.2.f.	proximal foramen of second tarsus.
ta.2.f'.	distal foramen of second tarsus.
ti.	tibia.
ti.f.	proximal tibial foramen.
ti.f'.	distal tibial foramen.
tr.	first trochanter.
tr.f.	trochanteral foramen.
tr.m.	trochanteral membrane.
tr.scl.	larger trochanteral sclerite.
tr.scl'.	smaller trochanteral sclerite.
v.	ventral.
1.	median tergo-conjunctival muscle.
2.	lateral tergo-conjunctival muscle.
3.	tergocoxopleural muscle.
4.	tergosternal muscle.
5.	anterior sternocoxopleural muscle.
6.	posterior sternocoxopleural muscle.
7.	anterior median levator.
8.	posterior median levator.
9.	superior external levator.
10.	inferior external levator.
11.	depressor.
12.	abductor.
13.	adductor.
14.	median flexor of femur.
15.	lateral flexor of femur.
16.	median levator of femur.
17.	lateral levator of femur.
18.	median flexor of tibia.
19.	lateral flexor of tibia.
20.	median levator of tibia.
21.	lateral levator of tibia.
22.	lateral flexor of first tarsus.
23.	lateral levator of first tarsus.
24.	flexor of claw.
25.	median retractor of genital sternite.

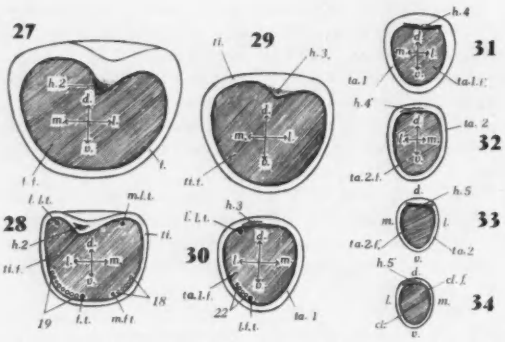


FIG. 27.—Posterior view of the right femur, showing its distal foramen.

FIG. 28.—Anterior view of the right tibia, showing its proximal foramen.

FIG. 29.—Posterior view of the right tibia, showing its distal foramen.

FIG. 30.—Anterior view of the right first tarsus, showing its proximal foramen.

FIG. 31.—Posterior view of the right first tarsus, showing its distal foramen.

FIG. 32.—Anterior view of right second tarsus, showing its proximal foramen.

FIG. 33.—Posterior view of the right second tarsus, showing its proximal foramen.

FIG. 34.—Anterior view of the right claw, showing its foramen.

DISCUSSION

The point arousing greatest interest regarding the anal legs of *Scolopendra* is the fact that these appendages do not take part in walking. The moving centipede keeps them lifted up from the ground, and the usual backward and forward strokes seen in other legs are absent. No sooner does the animal come to rest than the posttibial part is flexed downward so that the claws touch the ground and continue to be in that position, the vertical plane of the resting anal leg being more or less parallel with the body axis.

Correlated with the inability of the anal legs to participate in locomotion are a number of modifications met with in their skeletomuscular mechanism, notwithstanding the fact that the appendages still retain the leglike appearance, and the general segmentation of the telopodite follows the usual pattern.

Despite the fact that the coxopodite of the actual walking legs (those preceding anal legs) has become implanted in the membranous pleural wall, it still remains recognizable in the form of various coxal sclerites. In the 21st leg-bearing segment, the coxal and pleural components have completely fused into a tough coxopleural plate, providing thereby an excellent fulcrum for the powerful coxopleuropedal muscles. The anal legs, which are much longer than the walking legs, would not be well adapted to rapid walking in the shallow crevices where the Scolopendromorph centipedes are supposed to carry out their hunting and feeding. The shift in the origin of these legs from the lateral to the posterior side of the segment, therefore, while rendering them unfit to contribute any locomotory force, makes them more suitable to be brought quite close and parallel to the body axis so that they can be dragged along and thus in no way interfere with the speed.

The Y-shaped sclerotization on the anterior face of the walking legs connecting the coxa with the telopodite as described by Manton (1958, 1958a) is not seen in the anal legs although the position of articulation between the coxopleuron and the telopodite remains basically on the anterior (lateral) face of the leg. Besides, the absence of annular sclerotization passing around the proximal rim of the trochanter makes this single pivot articulation obviously too weak to permit the long and robust legs to be locomotive, although it is indeed good enough to allow rapid levation and abduction movements of the telopodite as a whole. The efficiency of such movements is dependent mainly on the shape of the prefemur (or trochanteroprefemur) and its extrinsic musculature. The ventrolateral flattening of the prefemur facilitates its upward and outward movement when the leg as a whole is worked upon by its extrinsic levators.

The increase in the number of extrinsic muscles is correlated with the increase in speed: most diplopods possess only two such muscles, while there may be as many as 18 in the running Scolopendromorpha and 33 in the fleet-footed centipede *Scutigera*. (See Manton loc. cit.). It is indeed interesting to note that the extrinsic musculature of the nonpropulsive anal leg in *Scolopendra* is reduced to seven muscles only.

The intrinsic musculature of an anal leg in *Scolopendra* consists of 11 muscles. The muscular disposition is quite in line with the movements actually performed by the anal legs. Manton has referred to these appendages as "posterior antennae" and hooks. The powerful flexor, whose origin extends right to the proximal rim of the prefemur, does certainly make them efficient hooks. Remington (1950) noticed *Scolopendra subspinipes* fastening its powerful anal legs near the ventilator hole of the tent peak and swinging its body quickly from side to side to seize insects that alighted nearby, attracted by the electric light.

The extrinsic levators of the leg are attached to that part of the first trochanter which is immovably joined to the prefemur, thereby effecting rapid and adequate levation of the leg. Similarly, the levators of femur, tibia, and first tarsus are directly inserted on these segments. The flexor muscles of all these segments are attached to somewhat mobile sclerites, thereby increasing the ease of flexure so essential for manipulative movements when the anal legs are used as hooks.

While the animal is on the move, the anal legs are kept lifted upward and outward by the powerful extrinsic levators, apparently behaving as "posterior antennae." The intersegmental movements con-

sist mostly of levation or depression. While side-to-side movements do occur between trochantero-prefemur and femur, femur and tibia, and tibia and tarsus, they are completely absent between the tarsal segments and at the tarsopretarsal junction, where the movements take place only in the vertical plane. Accordingly, the femur and tibia, together with their proximal and distal foramina, are dorsoventrally compressed while the compression is distinctly latero-medial in respect of the posttibial segments (figs. 26-34). The intersegmental movements of abduction are more marked than those of adduction as would be evinced by more robust muscles on the lateral side. There being no medial flexor of the tarsus, the posttibial part of the leg never performs any movement of adduction. While the femur, tibia, and first tarsus have each their levators situated in the preceding segments, the second tarsus and the claw have none, and the part of the leg beyond the first tarsus remains, therefore, somewhat bent downward when the leg as a whole is lifted up during locomotion (fig. 7). The hinge joints become more and more transversely extended distally so that the intersegmental movements, particularly those of levation, become correspondingly limited. Consequently, the muscles undergo reduction in their number and size: the levators do show clear size-reduction and the first tarsus has no levator or abductor muscle on the medial side at all.

ACKNOWLEDGMENTS

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Observations on the Subgenus *Argas* (Ixodoidea, Argasidae, *Argas*). 5. Study of *A. brevipes* Banks, 1908, from Birds in Arizona and California, U.S.A., and Baja California, Mexico[1]

GLEN M. KOHLS,[2] HARRY HOOGSTRAAL,[3] AND CARLETON M. CLIFFORD[2]

ABSTRACT

That this is a valid species (not a synonym of *A. r. reflexus* (F.)) has been confirmed by study of recently collected specimens, plus examination of the one remaining, though much mutilated, cotype. The inadequate original description was based in part on specimens from a bird nest in Saguaro cactus (*Carnegiea gigantea*) at Tucson, Pima Co., Arizona. Adults and nymphs are here recorded from such habitats in Pima and Maricopa Cos., Arizona, and in Baja California. Larvae are recorded from two species of owls and two of woodpeckers in Arizona, and from Sparrow Hawk in Alameda Co., California. In Baja California, adults and nymphs were found in a swallow nest in a Cirio tree (*Idria columnaris*). The adults and immature stages are described; characters for distinguishing them from other species of the subgenus are given; and preliminary biological data, obtained from rearing limited numbers of *A. brevipes*, are presented.

Argas brevipes Banks, 1908, was very briefly described from three specimens from Tucson, Arizona, two of them being from "a cavity in *Cereus giganteus*, which was used, perhaps, by a bird." The source of the third specimen was not specified. Although the sex of the specimens was not given, Banks' statement "Length of swollen female, 5 to 7 mm." infers that at least two were females. Cooley and Kohls (1944) regarded the species as doubtful because Banks' description was inadequate and the types could not be found, despite a search which included an inspection by Dr. J. C. Bequaert in 1940 of the col-

[1] From Research Project MR005-1402.03, Bureau of Medicine and Surgery, Navy Department, Washington 25, D. C. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. Accepted for publication March 24, 1961.

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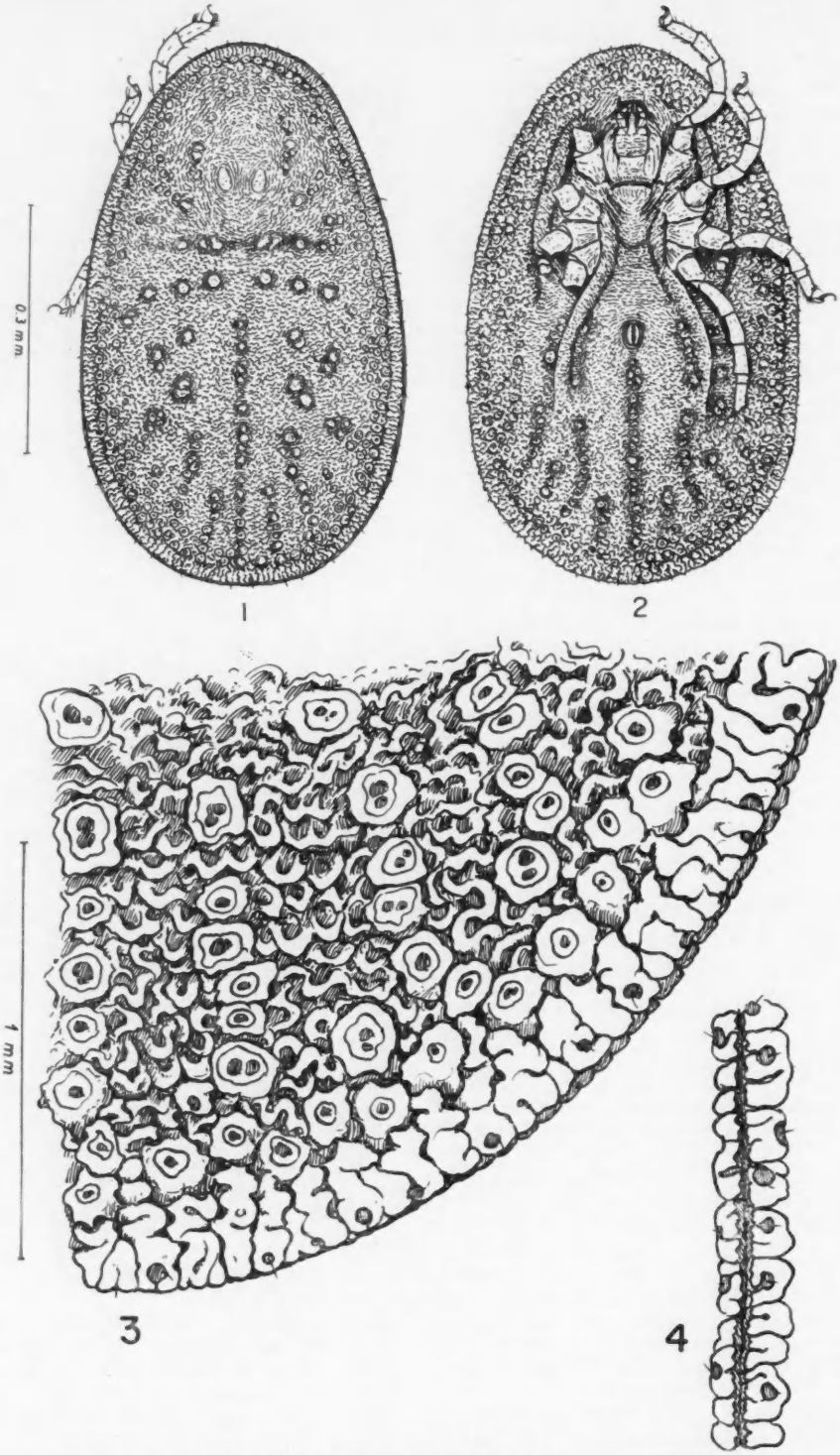
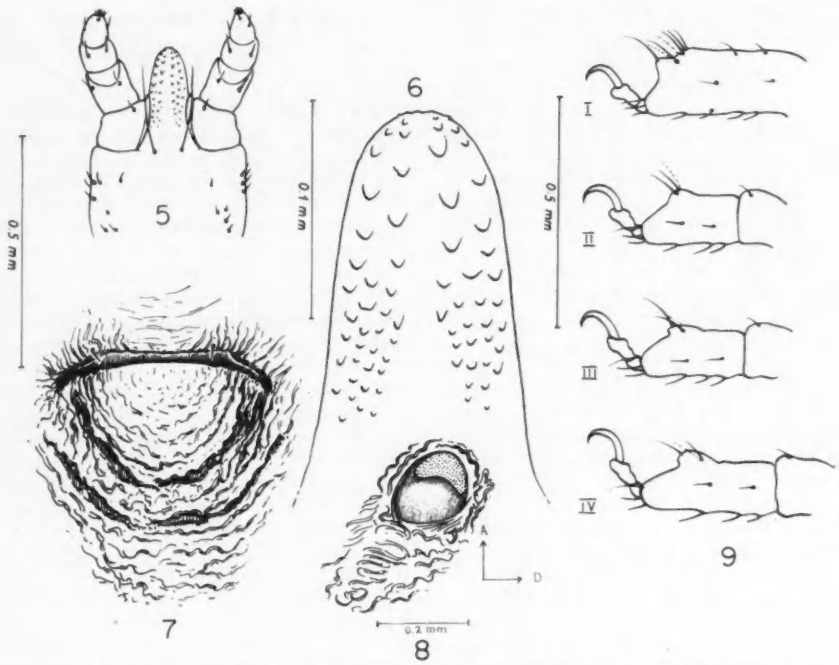
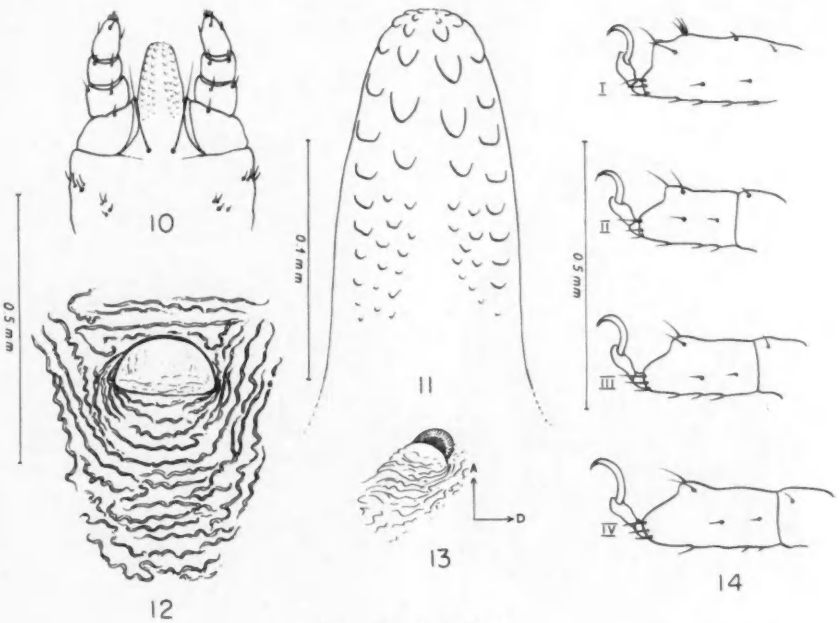


Argas brevipes Banks, female, RML 34767. FIGS. 1-2.—Dorsal and ventral views; FIGS. 3-4.—Integument, dorsal posterior quadrant, and integument, lateral view.



Argas brevipes Banks, female, RML 34767. FIGS. 5-6.—Capitulum and hypostome, ventral views; FIGS. 7-8.—Genital area and spiracular plate; FIG. 9.—Tarsi I-IV, lateral view.



Argas brevipes Banks

FIGS. 10-14, male, RML 35660. Figs. 10-11, capitulum and hypostome, ventral views; figs. 12-13, genital area and spiracular plate; fig. 14, tarsi I-IV, lateral view.

lection of the Harvard University Museum of Comparative Zoology where the specimens were presumed to have been deposited. Later Dr. Bequaert found a cotype in the MCZ and stated (1946) that *A. brevipes,* according to this specimen, is a synonym of *A. reflexus* (Fabricius, 1794). He sent the specimen to Dr. R. A. Cooley who stated (correspondence, 1946) that it should for the present be referred to *reflexus* in spite of some differences in size, length of legs, and body surface structures. The western North American *Argas* found principally in association with cliff swallows and doubtfully determined as *A. reflexus* by Cooley and Kohls (1944), has recently been described as a new species, *A. cooleyi* Kohls and Hoogstraal (1960).

Study of recently available preserved specimens from Baja California, Mexico, and Arizona, and especially of living specimens from near Tucson, Pima County, Arizona, for rearing, has revealed that *A. brevipes* is a valid species distinct from the two previously known North American species of the genus, *A. cooleyi* and *A. persicus* (Oken, 1818). Through the courtesy of Dr. H. Levi of the Museum of Comparative Zoology, we have been able to compare our material with the *A. brevipes* cotype, a female 5.43 mm. in length and lacking the hypostome and all legs except a portion of leg I, and could find no differences. Females, males, nymphs and larvae of *A. brevipes* are described below and preliminary biological data obtained from rearing a limited number of specimens through one generation at the Rocky Mountain Laboratory are given. Further biological information now being obtained will be presented in a separate report.

Argas (Argas) brevipes Banks, 1908

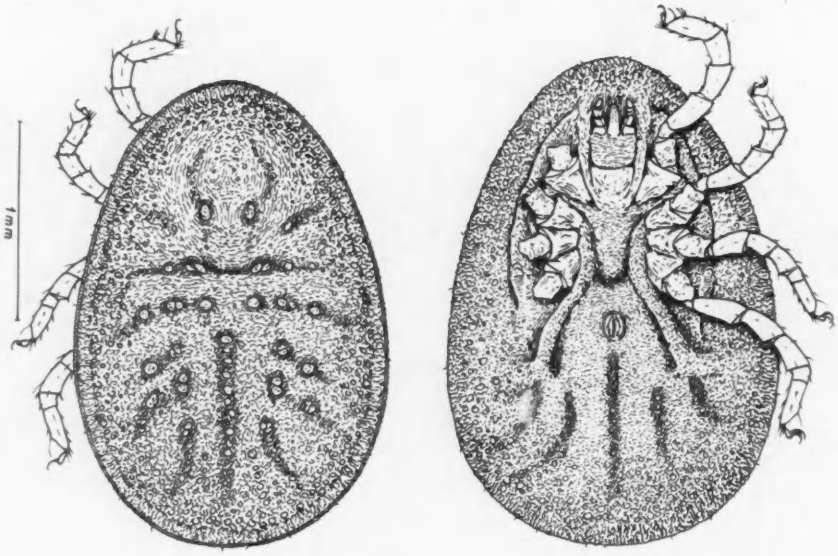
The Short-Legged North American Bird Argasid

(Figures 1 to 25)

REDESCRIPTION.—FEMALE (figs. 1-9). Measures from 3.69 mm. to 5.64 mm. in length and from 2.46 mm. to 3.59 mm. in width. Body (unengorged) thin and flat, concealing capitulum and most segments of legs; outline oval as illustrated.

Dorsal integument posteriorly (fig. 3) consists of numerous curved, flat-topped ridges of varying width. Anteriorly on body, elevations are smoother and form a definite convergent pattern among discs. *Setae* inconspicuous, fine, pale, widely and irregularly scattered among integumental elevations. *Discs* large and distinct, arranged in clearly radiating rows. *Lateral integument* (figs. 3-4) differentiated by a striated margin; scattered setae arising from pits as illustrated; *lateral suture* (fig. 4) distinct, encircling entire body. *Ventral integument* (fig. 2) with heavier elevations laterally, forming narrow, transverse ridges in intercoxal area; discs arranged as illustrated. *Hood* absent.

Capitulum (figs. 5-6) rather small, situated in shallow camerostome between coxae I. *Basis capituli* ventrally (fig. 5) rectangular; bearing three or four small basolateral setae, four small lateral setae on each side, one pair of very small submedian postpalpal bristles, another pair of long posthypostomal bristles arising level with and in close proximity to each palpal base and extending approximately to basal level of palpal segment 3. *Hypostome* (fig. 6) short and stout, slightly over twice as long as width at midlength of shaft, lateral margins slightly



15 16

Argas brevipes Banks, nymph, RML 34767. FIGS. 15-16.—Dorsal and ventral views.

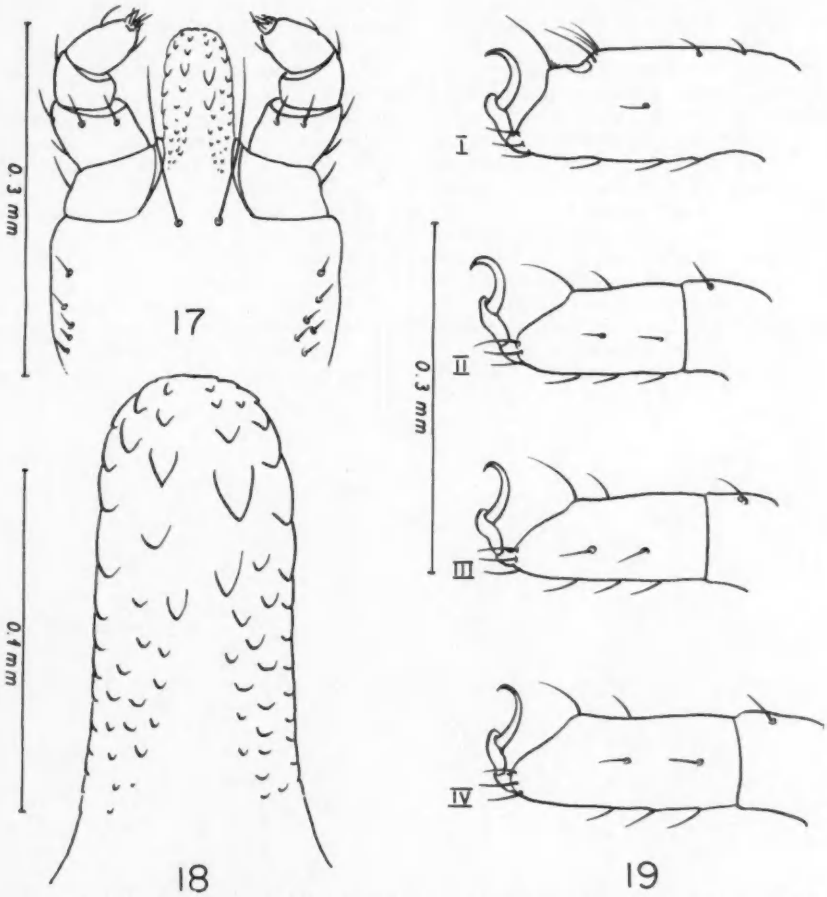
converging, apex gradually rounded and bearing only a slight suggestion of coronal hooklets; dentition consisting of comparatively short, bluntly rounded denticles, anteriorly 2/2, posteriorly smaller and 3/3 to 4/4. *Palpi* extending beyond hypostome by full length of segment 4 and partial length of segment 3; segments subglobose, decreasing in width from 1 to 4, segments 1 and 2 subequal in length, 3 and 4 shorter and subequal.

Eyes absent. *Spiracular plates* (fig. 8) small, situated laterad of coxae IV. *Genital aperture* (figs. 2, 7) forms a transverse slit at level of base of coxae I. *Anus* elliptical, nearly centrally located on venter, several fine setae arising from each valve. Ventral *"paired organ"* absent. *Coxal* and *supracoxal folds* present.

Legs (fig. 2; see also plate I, figs. 3 and 4, of Banks, 1908) remarkably short, length of leg IV excluding coxa about one-third that of body; coxae anterior to midlength of body, II to IV contiguous, I and II separated by a narrow integumental area. *Tarsi* (fig. 9) short and stout; II to IV usually with a mild subapical dorsal protuberance; dorsoapical margin of III and IV usually slightly retrograde, that of leg I more or less truncate; tarsi with setae visible from lateral view on each leg, from I to IV, typically as follows: apicodorsal 2/3, 2/3, 2/3, 2/3; subdorsal 2, 0, 0, 0; median 2, 2, 2, 2; apicoventral 3, 3, 3, 3; subventral 4, 3, 3, 3 (additional tuft of approximately three setae around Haller's organ). *Claws* small, subequal; *pads* (pulvilli) obsolete.

MALE (figs. 10-14). Similar to female except for smaller size and slight secondary sexual characteristics. Measures from 3.38 mm. to 4.61 mm. in length and from 2.05 mm. to 2.97 mm. in width. *Genital aperture* and *spiracular plate* as illustrated (figs. 12, 13).

Capitulum (figs. 10, 11). *Hypostome* similar to that of female, though corona may be slightly more



Argas brevipes Banks, nymph, RML 34767. FIGS. 17-18.—Capitulum and hypostome, ventral views; FIG. 19.—Tarsi I-IV, lateral view.

distinct. *Basis capituli* similar to that of female except that postpalpal bristles are either absent or incorporated into setal group. *Legs* similar to those of female except that subapical dorsal protuberances of tarsi are usually somewhat less elevated and more rounded.

NYMPH (figs. 15-19). Large nymphs may equal females in size and have a depression in place of the genital aperture. Nymphs of each instar resemble adults except for finer integument, slight differences in chaetotaxy of capitulum, slightly wider body outline (especially in first instar), relatively longer legs and absence of subapical dorsal protuberances on tarsi. The basis capituli bears about five to seven lateral setae on each side and a pair of posthypostomal bristles reaching almost to apex of the hypostome. The hypostome has parallel lateral margins, a bluntly rounded apical margin, 2/2 rows of comparatively large denticles anteriorly, 3/3 rows of smaller denticles basally, and a very slight corona.

LARVA (figs. 20-24, reared material from Arizona, RML 35660). Body, unengorged, subcircular, measuring approximately 0.45 mm. to 0.60 mm. in diameter (excluding capitulum); size when engorged from about 1.39 mm. to 1.61 mm. in length by 1.01 mm. to 1.19 mm. in width. Capitulum anterior. An ovate squamous area present on dorsal integument.

Setae of body and legs moderately long, lightly and inconspicuously fringed (fig. 20A), dorsally numbering seven anterolateral pairs, eight posterolateral pairs (total of 15 pairs of dorsolateral setae), two pairs beside squamous area, and seven setae on each posterior quadrant. Ventrally, with two setae on each coxa, three intercoxal pairs, three circumanal pairs, and one pair from anal valves.

Capitulum. Basis capituli with two pairs of minute bristles on ventral surface. *Hypostome* with lateral margins parallel, apex bluntly rounded or slightly indented, moderately elongate, approximately three times as long as wide, with denticle rows extending full length of shaft. Dentition 2/2, each row consisting of seven to nine denticles. Palpi with segments 1 to 3 subequal, 4 longer, with a single fringed seta arising from ventral surface of 2, 3, and 4.

Legs moderately long and stout; coxae each with two setae; *claws* moderately long; *pads* (pulvilli) reduced.

MATERIAL EXAMINED.—Two ♀, four nymphs from nest of Violet-green Swallow (*Tachycineta thalassina lepida*) in Cirio tree (*Idria columnaris*), 28 miles south of Punta Prieta, Baja California, Mexico, July 2, 1957, R. E., A. E., and J. V. Ryckman, and D. Spencer (RML 34753).

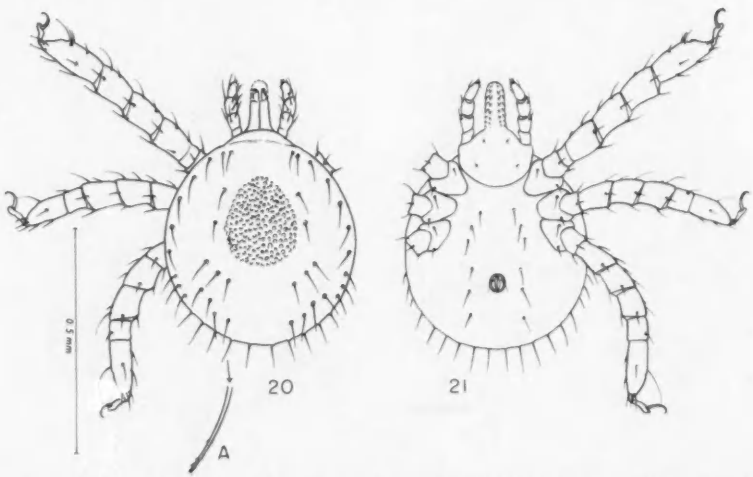
Two ♂, four ♀, three nymphs from woodpecker holes in Saguaro cactus (*Carnegiea gigantea*), 5.5 miles south of San Felipe, Baja California, Mexico, December 24, 1957, C. P. Christianson (RML 34767).

One ♂, two ♀, ten nymphs from woodpecker holes in Saguaro cactus, 19 miles east of Aguila, Maricopa County, Arizona, February 10, 1959, R. E. Ryckman and E. T. Ryckman (RML 34766).

Nine ♂, two ♀, 21 nymphs from nest, probably of Cactus Wren, *Campylorhynchus brunneicapillus couesi*, in holes near top of Saguaro cactus, 1 mile west of Arizona-Sonora Desert Museum, Tucson Mountain Park, Pima County, Arizona, January 3, 1960, H. Hoogstraal, F. G. Werner, and L. W. Walker (RML 35660). Larvae, nymphs and adults have been reared from this lot. Figure 25 shows habitat area and technique of collecting bird nests from holes in Saguaro cactus.

One larva from Ferruginous Pygmy Owl, *Glaucidium brasilianum*, near Tucson, Pima County, Arizona, January 6, 1948, A. R. Phillips (RML 28661).

One larva from Mearns' Gilded Flicker, *Colaptes*



Argas brevipes Banks, larva (unengorged, reared), RML 35660. FIGS. 20-21.—Dorsal and ventral views (A, posterior seta enlarged).

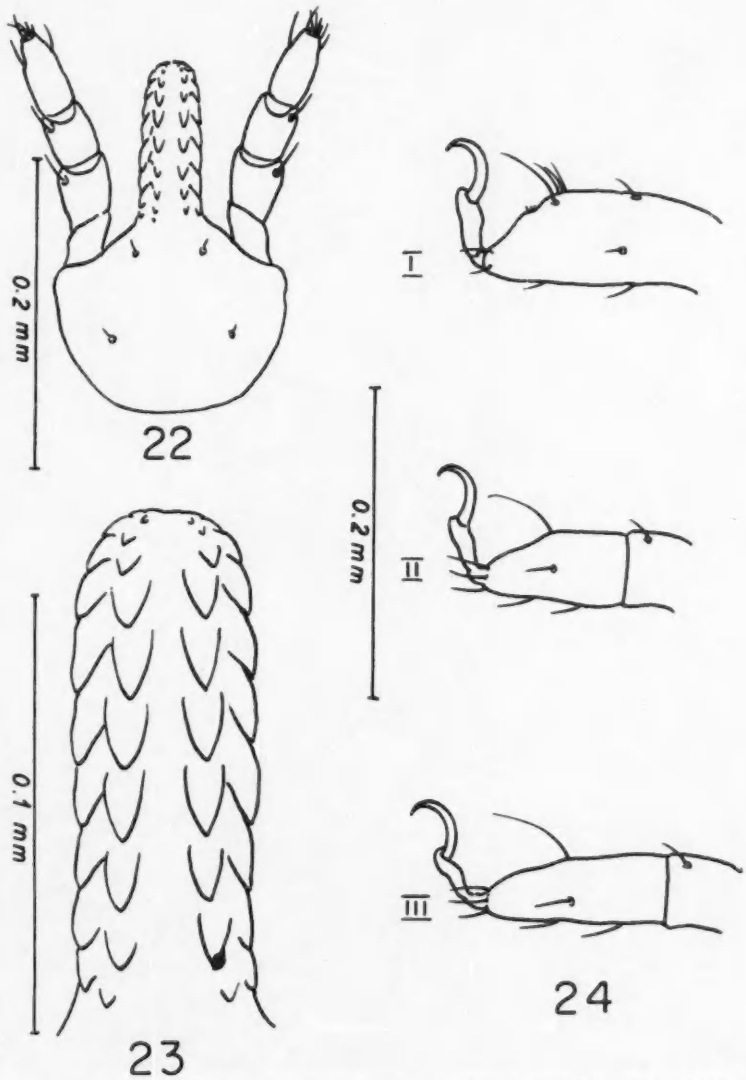
chrysoides mearnsi, Sahuarita Butte, near Tucson, Pima County, Arizona, July 7, 1938, A. R. Phillips (RML 28668).

One larva from Mearns' Woodpecker, *Balanosphyra formicivora aculeata*, near Tucson, Pima County, Arizona, July 15, 1939, A. R. Phillips (RML 28658).

Thirteen larvae from Screech Owl, *Otus asio*, mouth of Trout Creek, Big Sandy Valley, Mohave County, Arizona, October 3, 1949, A. R. Phillips (RML 28652 and 28660).

Two larvae from a collection of several larvae from Sparrow Hawk, *Falco sparverius*, Alameda County, California, March 30, 1960, D. P. Furman (RML 36851); also two ♀ and two nymphs reared from this lot.

RELATED FORMS.—*Argas brevipes* adults resemble those of *A. cooleyi* (western North America) and *A. r. reflexus* (Europe) but are distinguished from these and all other members of the subgenus *Argas* by their small size; short legs, which are largely concealed by the body; short, blunt tarsi with rather rounded subapical dorsal protuberances; and by the dentition of the hypostome, which is *2/2* anteriorly



Argas brevipes Banks, larva (unengorged, reared), RML 35660. FIGS. 22-23.—Capitulum and hypostome, ventral views; FIG. 24.—Tarsi I-III, lateral view.

and 3/3 to 4/4 posteriorly, the teeth being bluntly rounded and comparatively short.

The larvae of *A. brevipes* bear comparatively few, lightly fringed setae; dorsally these typically number 15 pairs laterally, 2 pairs around the squamous area and 7 setae on each posterior quadrant. For the larva of *A. cooleyi* these totals are typically 22, 1, and 9, while for *A. neghmei* Kohls and Hoogstraal (1961) (northern Chile) they are 20, 2, and 6. Respective totals for *A. r. reflexus* are 26, 1, and 9;

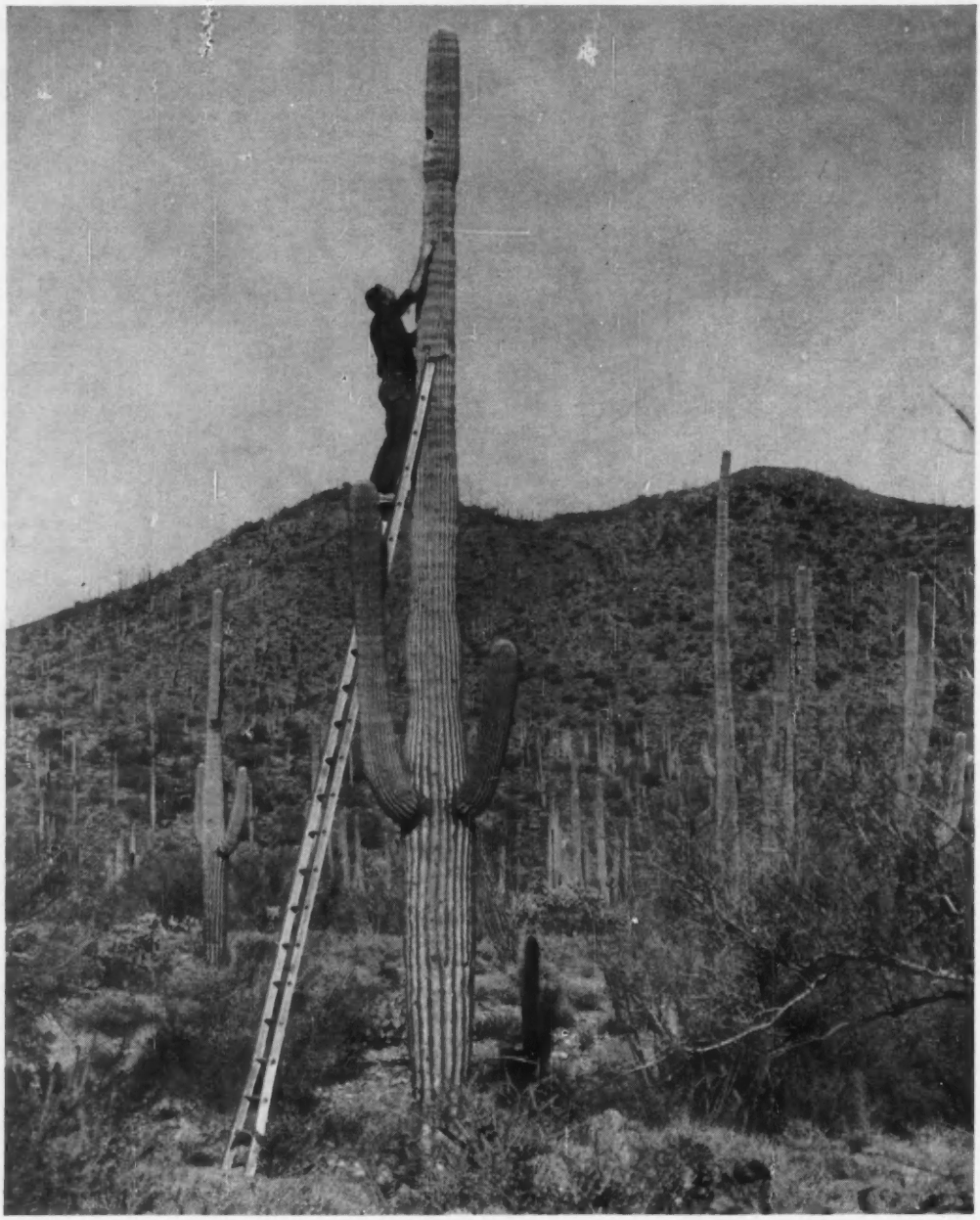


FIG. 25.—View of habitat area of *A. brevipes* near Tucson, Arizona, showing technique of collecting bird nests from holes in Saguaro cactus. Photo courtesy Dr. W. H. Woodin, Arizona-Sonora Desert Museum.

for *A. r. hermanni* Audouin, 1827 (Africa) are 15, 1, and 7; and for *A. persicus* (cosmopolitan) are 15, 3, and 7 plus 3 anterior submedian pairs. Thus far, the larvae of only *A. neghmei* and *A. brevipes* are known to have two pairs of setae beside the squamous area. The squamous areas of these two species differ only slightly, that of *A. brevipes* being slightly wider than that of *A. neghmei*. In these respects the larva of *A. brevipes* appears to be more like *A. neghmei* than *A. cooleyi*. Additional features for species differentiation of larvae of the genus *Argas* are given by Sonenshine, Clifford and Kohls (in press).

DISTRIBUTION AND HOSTS.—*A. brevipes* is recorded from Arizona and California, U.S.A., and Baja California, Mexico. Hosts of the larvae include Screech Owl, *Otus asio*; Ferruginous Pygmy Owl, *Glaucidium brasilianum*; Mearns' Gilded Flicker, *Colaptes chrysoides mearnsi;* Mearns' Woodpecker, *Balanosphyra formicivora aculeata*; and Sparrow Hawk, *Falco sparverius*. All of these birds build their nests in hollow trees or holes made in trees or Saguaro cactus. Adults and nymphs of *A. brevipes* have been found only in nest cavities in the Saguaro cactus and the Cirio tree. In one instance the nest was that of a Violet-green Swallow, *Tachycineta thalassina lepida* and in another the nest was presumably that of a Cactus Wren, *Campylorhynchus brunneicapillus couesi*.

BIOLOGY.—Preliminary life-history data were obtained by rearing specimens (RML 35660) collected in January 1960 in Arizona. Adults, nymphs and larvae fed readily on week-old chicks. Adults and nymphs fed to repletion in from about 10 minutes to an hour, and larvae in from 4 to 8 days. At 30° C., larvae moulted to nymphs in 3 to 5 days, and nymphs, of which there are three or four instars, moulted in about 7 days after each feeding. Females began oviposition about a week after feeding and produced up to approximately 50 eggs over a period of several days after each meal. Some of our females have produced as many as four batches of eggs thus far. Hatching began about 10 days after the first eggs were deposited and larvae fed readily a week or so after hatching. Some adults still alive have survived for about a year at room temperature without feeding.

ACKNOWLEDGMENTS

We are grateful to Dr. Floyd G. Werner for the opportunity to study the argasid material in the University of Arizona collections. He and Mr. Lewis W. Walker of the Arizona-Sonora Desert Museum, Tucson, made arranagements and provided equipment used in obtaining living specimens of this species. Dr. W. H. Woodin, Director of the Museum, provided the photograph reproduced as figure 25. Dr. Raymond E. Ryckman, of the College of Medical Evangelists, Loma Linda, California, kindly placed at our disposal the specimens collected by him and his associates in Baja California and Arizona, and advised us as to the currently accepted technical names of the Saguaro cactus and the Cirio tree. The California specimens were received through the courtesy of Dr. Deane P. Furman, University of California, Berkeley. We also wish to thank Dr. H. Levi, of the Museum of Comparative Zoology, Harvard University, for sending us the *A. brevipes* cotype for comparison.

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Heterosis in the Honey Bee as Shown by Morphological Characters in Inbred and Hybrid Bees[1]

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ABSTRACT

Inbred queens from eight lines representing several races of honey bees (*Apis mellifera* L.) were mated (artificially inseminated) to drones of the same line and also to another line, so that inbred and hybrid worker offspring were produced simultaneously from each mother queen. By the use of genetic markers the two types of offspring were identifiable. Small but highly significant differences between lines were found in the characteristics wing length, wing width, cubital index, number of wing hooks, tongue length, and antenna lengths. The hybrid bees were usually larger than their inbred sisters. This difference in size suggests that hybrid vigor is present in honey bees. Uncontrollable feeding variability between colonies apparently accounted for significant differences in some samples.

Practical control of the breeding of honey bees (*Apis mellifera* L.) originated with Watson's (1927) description of a method of artificial insemination of queens. Since then many workers have made important contributions to the knowledge of bee breeding. This progress has been reviewed by Roberts and Mackensen (1951), Ruttner and Mackensen (1952), Kerr and Laidlaw (1956), and Rothenbuhler (1958).

The success of hybridization with other animals and plants and the undesirability of line breeding in honey bees because of the presence of certain lethal alleles (Mackensen 1951) suggested a hybrid breeding program for honey bee improvement. Hybrid vigor in honey bees has been indicated in tests based on the amount of honey produced and the ovulation rate of queens (Cale and Gowan 1956). The influence of heredity on honey production is difficult to measure owing to the many other factors that cannot be readily controlled.

In 1955 experiments were conducted at Baton Rouge, La., to investigate the difference, if any, in readily measurable morphological characteristics of inbred and hybrid honey bees. If differences could be shown and heterosis could be demonstrated, other differences could be expected to occur in characteristics not readily measurable, the most important of which are honey production, vigor, and longevity. Schwartz (1953) had found heterosis in morphological characteristics, but in such small amounts that a more precise experimental design was needed to estimate the relative importance of the the variables concerned.

MATERIALS AND METHODS

Eight inbred lines representing several races or strains of bees were used. The lines S, L, and M originated from Italian bees, F and Z from Caucasian, R and Y from Golden Italian, and X from dark-banded English bees. Inbreeding coefficients of these lines were as follows: S, 0.73; F, 0.54; Z, 0.73; R, 0.62; X, 0.51; Y, 0.79; L, 0.49; and M, 0.82. Only lines R and Y were known to be related to each other, in this instance a 50% relationship. Changes in homozygosity and relationship were estimated by formulas given by Crow and Roberts (1950).

Queens of each line were mated (artificially inseminated) to one drone of the same line and one drone of another line, so that inbred and hybrid worker offspring were produced simultaneously from each common mother. Matings of black and yellow body-color lines produce banded intermediate hybrids; cordovan is recessive to black body color and chartreuse is recessive to wild-type eye color, thus these matings produce wild-type offspring. By the use of these genetic markers it was possible to separate the half-sister offspring of each queen. Since only a limited number of marker genes was available the hybrid crosses were selected on the basis of these genetic markers rather than racial origin or relationship of the lines. The crosses were therefore not random but previously determined by feasibility in separation of half-sisters. All crosses were made in reciprocal.

Honey bees feed their young glandular and selected foods of their own choice. The primary sources of these foods are pollen and nectar from plants. Nectar is converted into honey and both nectar (or honey) and pollen are necessary for the production of the glandular foods. The feeding and care of the young are affected not only by the availability of food but also by the number of nurse bees and of immature young. In an attempt to minimize rearing differences and to measure their effects, if any, special feeder colonies were used for brood rearing. All feeder colonies contained populations of approximately 30,000 bees, comparable amounts of brood, and adequate amounts of honey and pollen.

To analyze the factors affecting the differences in the offspring from the experimental lines and crosses, a Latin-square design was used. Latin square 1 is shown on the following page.

In the Latin square diagram the feeder colonies correspond to rows, the periods to columns, and the matings to treatments. The interval between egg-laying periods was 2 weeks, and the tests began on June 27. Two inbred lines and their reciprocal hybrids were reared simultaneously in each of four cells of the Latin square. Each rearing cell within this Latin square therefore contains four mating

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[2] In cooperation with Louisiana State University.

PERIODS

COLONIES	1	2	3	4
1	SS SY YY YS	FF FR RR RF	ZZ ZL LL LZ	XX XM MM MX
2	FF FR RR RF	ZZ ZL LL LZ	XX XM MM MX	SS SY YY YS
3	ZZ ZL LL LZ	XX XM MM MX	SS SY YY YS	FF FR RR RF
4	XX XM MM MX	SS SY YY YS	FF FR RR RF	ZZ ZL LL LZ

types: for example, inbreds SS and YY and hybrids SY and YS. The design was thus four mating-type cells (treatments) produced once in each colony (row) and once in each period (column). These paired rearings permitted direct comparisons between mating types within cells. Eight inbreds and four hybrids in reciprocal comprised a Latin square. Three such experiments were conducted simultaneously.

Brood samples were obtained from eggs laid within a 24-hour period. Combs for egg laying had been previously used for not more than two cycles of brood. To avoid bias owing to possible differences between combs, the combs were exchanged between reciprocal pairs in the first and third, and second and fourth periods. The combs were given to the breeder-queen nuclei on specific dates. (A nucleus is a small colony of bees maintained in a small hive. It is equivalent to a normal colony in most aspects of organization and activity except size.) These combs, containing eggs, were removed on the next day and immediately placed between combs of unsealed brood above a queen excluder in the feeding colonies. Each feeder colony received two combs of eggs on each selected date. The colony fed and cared for the experimental bees throughout their development. On the 18th day of development the brood combs were removed from the feeder colonies, caged, and placed in an incubator until emergence of adults was completed. Emerged bees were removed from the cages twice daily (8 a.m. and 5 p.m.), killed with ethyl acetate fumes, and injected with preserving fluid for later morphological measurements.

Ten inbred and ten hybrid bees from each rearing comprised a sample for analysis of morphological variance of the progeny of each queen. Since the full sisters from the progeny of each queen had very high coefficients of relationship (0.87-0.95), a small number of siblings was deemed adequate to obtain a valid sample of the variability between sisters. Seven morphological characteristics were selected for study: (1) forewing length, (2) forewing width, (3) cubital index,[3] (4) number of wing hooks on hindwing, (5) tongue length (mentum and submentum combined), (6) antenna scape length, and (7) antenna flagellum length. One forewing and one hindwing, one antenna, and the tongue from each bee were mounted on glass slides, and the images projected on a calibrated screen for measurements. The number of wing hooks and the cubital index were determined under a binocular microscope. All measurements were made to the nearest 0.05 mm.

RESULTS AND DISCUSSION

Data obtained on these characteristics are presented in table 1. The averages are grouped by Latin squares and mating pairs. Although all measurements were made to two decimal places the averages are given to three places for the benefit of those readers who may elect to compute the mean squares for contrasts and perform a test of significance.

Latin square 1 consisted of pairings S vs. Y, F vs. R, Z vs. L, and M vs. X, as previously shown in the diagram. The next four pairings in the table comprised Latin square 2 and the last four pairings made up Latin square 3. The four mating type averages in each paired breeding cell are grouped

[3] The cubital index is the ratio of the two parts into which a line joining the two corners of the third cubital cell of the forewing on the side bounded by the cubital vein is divided when a perpendicular is dropped from this line to the second recurrent vein.

Table 1.—Paired comparisons of averages of morphological characteristics in inbred and hybrid worker honey bees.[a]

Matings		Paired rearings											Avg.
		Latin square 1				Latin square 2				Latin square 3				
		S vs Y	F vs R	Z vs L	M vs X	S vs L	Z vs M	X vs R	F vs Y	S vs F	Z vs R	X vs Y	L vs M	
Wing lengths														
Inbreds	A	9.268	9.108	9.205	8.996	9.113	8.949	8.813	9.036	9.213	9.014	8.891	9.293	
	B	9.068	9.349	9.206	8.903	9.338	9.103	9.251	9.046	9.144	9.291	9.068	9.005	9.104
Hybrids	AB	9.335	9.384	9.211	9.066	9.188	9.118	9.185	9.261	9.285	9.248	9.116	9.271	
	BA	9.349	9.275	9.259	9.164	9.406	9.184	9.231	9.206	9.351	9.284	9.146	9.215	9.238
IMS[b]		0.0279	0.0214	0.0129	0.0048	0.0095	0.0180	0.0451	0.0085	0.0141	0.0306	0.0269	0.4013	
Wing widths														
Inbreds	A	3.366	3.155	2.983	3.294	3.288	2.968	3.119	3.096	3.336	2.976	3.108	3.310	
	B	3.141	3.160	3.210	3.103	3.269	3.270	3.135	3.114	3.165	3.150	3.144	3.256	3.171
Hybrids	AB	3.308	3.221	3.189	3.230	3.283	3.194	3.156	3.208	3.328	3.080	3.165	3.334	
	BA	3.288	3.175	3.161	3.248	3.336	3.213	3.161	3.200	3.289	3.158	3.158	3.309	3.224
IMS[b]		0.0031	0.0041	0.0019	0.0010	0.0016	0.0026	0.0013	0.0019	0.0016	0.0053	0.0044	0.0052	
Cubital index														
Inbreds	A	2.200	2.457	1.751	3.056	2.138	1.913	3.318	2.386	2.298	1.855	3.435	2.142	
	B	2.784	1.875	2.202	3.304	2.275	2.847	1.679	2.642	2.207	1.725	2.410	2.764	2.402
Hybrids	AB	2.392	2.110	2.251	3.718	2.055	2.320	2.462	2.535	2.196	1.719	3.073	2.624	
	BA	2.578	1.897	2.048	2.979	2.016	2.369	2.664	2.388	2.075	1.838	2.986	3.122	2.434
IMS[b]		0.1115	0.0420	0.0559	0.1082	0.0280	0.0155	0.0825	0.0589	0.0696	0.0214	0.1911	0.0972	
Wing hooks														
Inbreds	A	20.98	21.65	22.03	20.03	21.10	21.95	20.80	21.35	21.85	21.63	21.25	21.50	
	B	20.08	19.18	21.65	20.23	21.60	19.13	19.83	20.23	21.45	19.43	20.58	20.63	20.84
Hybrids	AB	20.73	21.05	21.65	21.70	22.43	20.85	20.02	22.33	21.13	20.30	21.63	20.38	
	BA	21.05	21.08	21.88	20.75	20.45	20.90	20.18	22.30	20.78	21.40	21.65	21.50	21.17
IMS[b]		0.29	4.16	1.58	1.41	2.47	2.27	0.79	1.13	2.04	0.84	0.72	1.62	
Tongue lengths														
Inbreds	A	6.169	6.050	5.993	5.870	6.128	5.953	5.673	6.046	6.185	5.979	5.774	6.071	
	B	5.848	5.886	5.931	5.721	6.174	5.959	5.895	5.861	6.193	5.958	5.895	5.941	5.965
Hybrids	AB	6.193	6.144	6.103	5.950	6.131	6.110	5.884	6.148	6.431	6.058	5.915	6.114	
	BA	6.174	6.155	6.146	5.949	6.285	6.228	5.895	6.128	6.380	6.108	5.954	6.055	6.110
IMS[b]		0.0138	0.0383	0.0038	0.0106	0.0053	0.0098	0.0157	0.0049	0.0075	0.0085	0.0170	0.0048	
Antenna scape														
Inbreds	A	1.536	1.571	1.530	1.554	1.526	1.518	1.479	1.540	1.531	1.510	1.473	1.539	
	B	1.500	1.510	1.518	1.495	1.551	1.526	1.510	1.503	1.563	1.504	1.504	1.524	1.521
Hybrids	AB	1.533	1.571	1.550	1.543	1.528	1.549	1.496	1.555	1.565	1.521	1.496	1.564	
	BA	1.546	1.551	1.523	1.516	1.548	1.575	1.503	1.545	1.588	1.536	1.505	1.545	1.540
IMS[b]		0.0016	0.0038	0.0008	0.0016	0.0006	0.0008	0.0012	0.0009	0.0001	0.0006	0.0021	0.0041	
Antenna flagellum														
	B	3.045	3.101	3.101	3.026	3.010	3.095	2.861	3.044	3.020	3.070	2.881	3.098	
Inbreds	A	3.071	3.135	3.073	2.893	3.118	3.048	3.135	3.035	3.114	3.143	3.081	3.059	3.052
Hybrids	AB	3.100	3.123	3.093	3.016	3.021	3.078	3.016	3.113	3.104	3.115	3.009	3.039	
	BA	3.094	3.138	3.145	3.001	3.091	3.134	3.031	3.095	3.094	3.118	3.011	3.080	3.077
IMS[b]		0.0101	0.0137	0.0028	0.0059	0.0044	0.0045	0.0040	0.0034	0.0040	0.0078	0.0015	0.0026	

[a] All averages on rows A, B, AB, and BA in the table are in millimeters except cubital index (a ratio) and wing hooks (a number). A denotes the first and B the second of the two paired lines of bees in each column; AB and BA denote the reciprocal hybrids.

[b] Interaction mean squares.

by columns. The average for the first of the two paired lines shown at the top of the column is always given on line A and that for the second inbred is given on line B. The reciprocal hybrid averages are given on lines AB and BA. On line AB the first inbred is the female parent and on line BA the first inbred is the male parent of those reciprocal hybrids.

In the S vs. Y column the four samples of inbred SS bees averaged 9.268 mm. in wing length, and their half-sisters (SY hybrids) reared simultaneously averaged 9.335 mm. The four samples of inbred YY bees, reared in the same colonies at the same times, had an average wing length of 9.068 mm., whereas their half-sisters (YS hybrids), averaged 9.348 mm. in wing length. As can be seen in the F vs. R. column, the corresponding average wing lengths were FF 9.108 mm., FR 9.384 mm., RR 9.349 mm., and RF 9.275 mm. The average wing length for all inbreds was 9.104 mm. and for hybrids 9.238 mm. (Last column at right, table 1.)

In preliminary analyses the hybrids and inbreds were compared separately. To analyze for differences between hybrids, the observations for reciprocals were pooled, with one average in each colony for each period for each hybrid. These Latin square designs were analyzed for differences among hybrid means. Analyses of three such Latin squares showed significant differences between hybrids for each of the seven morphological characteristics, and thus demonstrated real genetic differences between hybrids.

Inbreds within a Latin square always occurred in pairs but in other Latin squares they appeared in combination with different lines. To approximate randomness the data for inbred lines from the three Latin squares were pooled for analyses. These analyses showed significant genetic differences between the inbred for each of the seven morphological characteristics. Because of the diversity of origin of lines, these genetic differences between inbreds and between hybrids were logical expectancies.

The foregoing analyses do not furnish direct evidence of heterosis. Such evidence may be obtained from comparison of the means of the inbreds and hybrids within rearing cells. Because they always occurred together in each of the four colonies, SS, YY, SY, and YS are a rearing cell. Individual paired comparisons within cells were made. The majority of the mean squares were

Table 2.—Analysis for wing length of the S vs. Y rearing cell of Latin square 1.

Source of Variation	d. f.	Mean Squares
Samples	3	0.0307 **
Matings	3	0.6702 **
Inbreds (A vs. B)	1	0.8000 **
Reciprocal hybrids (AB vs. BA)	1	0.0031
Inbreds vs. hybrids (A&B vs. AB&BA)	1	1.2076 **
Half-sisters (A vs. AB)	1	0.0911
Half-sisters (B vs. BA)	1	1.5680 **
Paired rearings (A&AB vs. B&BA)	1	0.3516 **
Samples x matings	9	0.0279 **
Error	144	0.0038
Total		

significant at the 1% level. The analysis for wing length of the S vs. Y rearing cell of Latin square 1 is given above.

The variance due to matings is the within-cell variance caused by differences between the four types of progenies within each cell. This variance was broken down into its three orthogonal components: (1) inbreds (A vs. B), (2) reciprocal hybrids (AB vs. BA), and (3) inbreds (A&B) vs. hybrids (AB&BA). The same mating variance within cells was also apportioned into another optional orthogonal analysis—namely, the three components (1) inbred SS (A) vs. hybrid SY (AB), (2) inbred YY (B) vs. hybrid YS (BA), and (3) progenies SS + SY (A&AB) vs. YY + YS (B&BA)—to permit comparisons between half-sisters produced simultaneously. The error term for testing the significance of the two sets of orthogonal comparisons of the progeny matings within cells was the interaction mean square of samples x matings.

All of the many individual comparisons possible from the averages given in table 1 have been computed[4]. Space limitations prevent their presentation in this paper. From the averages and interaction mean squares for each paired rearing cell in the table the reader may make the following orthogonal comparisons for each characteristic:

Contrasts	Means A	B	AB	BA
Set 1 Inbreds	1	−1		
Hybrids			1	−1
Inb. vs. Hyb.	1	1	−1	−1
Set 2 Half sisters A vs. AB	1		−1	
Half sisters B vs. BA		1		−1
Paired rearings A&AB vs. B&BA	1	−1	1	−1

Computations are as follows:
Mean squares contrasts
= 20 (difference between means)^2 for two mean contrasts
= 10 (difference between means)^2 for four mean contrasts

$$F = \frac{\text{Mean square contrasts with 1 d.f.}}{\text{Mean square Samples x Matings with 9 d.f.}}$$

In table 3 a summary analysis is presented. This analysis showed that both genetic and nongenetic factors contributed significantly to the variance between samples and between matings. Computations made but not shown indicated significant differences between replicated samples in one or more paired comparisons for all characteristics measured. Individual differences between feeder colonies accounted for the major part of this variance. Seasonal influences, as measured by periods, also contributed to significant differences in some characteristics.

In all paired comparisons for wing length, wing width, and tongue length the means of the hybrids were significantly greater than the means of the inbreds of the parental lines. Of these 36 comparisons, 35 were significant at the 1% level and one (L vs. M for wing length) was significant only at the 5% level. Heterosis was therefore demonstrated. For the antenna characteristics and the number of wing hooks heterosis was evident in some pairings but not in others. The cubital indices of the hybrids,

Table 3.—Summary analyses of variance of seven morphological characteristics in inbred and hybrid honey bees.

Variance Source	d.f.	Mean Squares: Wing length	Wing width	Cubital index	Wing hooks	Tongue length	Scape length	Flagellum length
Latin squares	2	0.1886	0.0485*	1.6797**	1.92	0.3506**	0.0067*	0.0360
Colonies within Latin square	9	0.4255**	0.0542**	0.2324	4.73	0.1256**	0.0069**	0.0286
Between matings within Latin Square	9	1.3201**	1.0230**	33.2322**	41.14**	3.1818**	0.0761**	0.3960**
Periods	3	0.4160*	0.0285	0.4137	16.77**	0.2099**	0.0163**	0.0434*
Periods X Latin Square	6	0.1180	0.0091	0.2979	1.11	0.0495	0.0015	0.0086
Error (a)	18	0.0991	0.0132	0.2273	2.57	0.0336	0.0016	0.0135
Within mating groups	36	0.6394**	0.2161**	4.2880**	23.43**	0.4951**	0.0164**	0.1141**
Inbreds vs. inbreds	12	1.0036**	0.4919**	10.0887**	39.64**	0.4565**	0.0233**	0.2749**
Reciprocal hybrids	12	0.1457**	0.0252**	1.6946**	12.64**	0.0841**	0.0074**	0.0234**
Inbreds vs. hybrids	12	0.7689**	0.1314**	1.0808**	18.00**	0.9447**	0.0185**	0.0440**
Mating Group X Periods	108	0.0217**	0.0028**	0.0735	1.82	0.0117**	0.0015**	0.0054**
Error (b)	1728	0.0048	0.0010	0.0618	1.49	0.0026	0.0004	0.0012
Intraclass correlations		0.66	0.85	0.79	0.28	0.85	0.54	0.70

[4] A mimeographed copy of the individual comparisons will be made available to interested research workers upon request to the author.

although tending to be greater than the mean of their inbred parents, were generally intermediate between the parental means. Since the cubital index is not a measure of size but reflects the relative lengths of two parts of the cubital vein, this measurement would hardly be expected to show contrary results.

It is evident that superiority for greater size in one characteristic does not necessarily indicate a corresponding superiority in other characteristics. Inbred lines S, R, and L had the longest wings among inbreds, and S and L appeared in the top three for wing width and tongue length. However, line M, with below-average wing and tongue lengths, had wing width considerably above the mean of all inbreds. Inbred line F, with an average wing length and below-average wing width, was second highest in tongue length. Inbred line X was at or near last place in wing, tongue, and antenna length. Its high cubital index and the converse low index for lines Z and R indicate relative but not absolute wing-vein measurements.

The hybrid bees showed not only highly significant differences among themselves for all characteristics measured, but also an increase in size over the mean of their parents. In most measurements of wing and tongue lengths, they exceeded the mean of the larger parent by 1% to 3%. Hybrids having F as one parent had tongue lengths above the average for other hybrids and significantly above the mean of the longer-tongued parent. The hybrid daughters of inferior lines showed the greatest relative evidence of heterosis. This was particularly evident when the wing lengths of inbred and hybrid half-sister progenies of X and Z queens and the tongue lengths of progenies of X, Y, and M queens were compared.

In most of the reciprocal hybrids the means were similar. However, certain exceptions were evident, with highly significant differences in several crosses. In 8 out of 12 of the reciprocal hybrids at least one of the seven characteristics measured showed highly significant differences between reciprocals. The most extreme case was hybrid SL which was consistently below its reciprocal cross in wing length, wing width, tongue length, and both antenna measurements, but it had more wing hooks. Since it is not known whether the inbred lines were homozygous for all genes affecting these characteristics, and the inbred S bees from this queen were smaller than those from the other two S queens in Latin squares 1 and 3, it is possible that genetic factors were responsible for this smallness.

Since all reciprocals were produced simultaneously, it may be assumed that colony environmental factors were constant. However, the bees themselves, and not the beekeeper, regulate the environment and nutrition of the young. Although, from egg to adult, the environmental factors for reciprocals were constant in so far as it is presently experimentally possible with honey bees, the pre-oviposition factors were not constant, since the breeder queens were in separate nuclei. If pre-oviposition environment did not cause significant differences between reciprocals in these experiments and there was homozygosity within inbreds, then these differences might have been caused by maternal influences. Such influences would permit consistent superiority of one hybrid over its reciprocal. However, lack of homozygosity within lines and/or unknown feeder-colony influences may also account for what appeared to be maternal influences.

The mating plan of these experiments produced a high degree of genetic likeness within matings. This likeness did not insure equal genetic likeness between or within lines for any of the characteristics measured. However, the number of lines and crosses involved (48) was sufficiently large to indicate that intraclass correlations would give valid estimates of the heritability of these characteristics under the conditions of these experiments. The intraclass correlations are shown in table 3. Heritability was high for wing width, tongue length, and cubital index, slightly lower for wing, scape, and flagellum lengths, and decidedly low for number of wing hooks. Since the eight inbred lines of bees used in these experiments were not previously selected for differences in any of the characteristics measured, it is logical to suggest that these estimates of heritability are indicative of the relative importance of heredity and environment in the characteristics observed.

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The Control of Water Loss by the Two-Spotted Spider Mite (*Tetranychus telarius*)[1]

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ABSTRACT

The two-spotted spider mite regulates its water loss by virtue of a relatively impermeable cuticle and control of diffusion of water from the tracheal system by movements of the mandibular plate. The diapause form exhibits a reduction in oxygen consumption coupled with reduced water loss from the tracheal system. Water intake in the feeding mite equals up to one quarter of the total weight within 1 hour. Fluids are bypassed to the hindgut, where small ions can be absorbed, and the water rapidly eliminated. Diffusion from the trachea can account for a large part of water excretion under conditions of low relative humidity.

The ratio of surface area to volume imposes a severe water stress upon any animal the size of a mite whenever it encounters a vapor pressure deficit. Although on the leaf surface, the usual environment of the mite, the relative humidity is usually high, increasing logarithmically as the leaf surface is approached (Frampton and Longrie 1941) and webbing produced by the mites has the further effect of maintaining the humidity gradient above the leaf, the mite must be able to maintain its water balance during unfavorable conditions. Boudreaux (1958) has shown a direct relationship of egg production, and indirectly feeding rate, to depend upon the relative humidity surrounding the food plant. From his work it can be assumed that the rate of water excretion governs the feeding rate, on which egg production depends, and that the high water content of the food presents a problem in excretion rather than conservation.

However, under nonfeeding and premolt conditions, the mite must be able to conserve water, as it does not live in a truly aquatic environment. Blauvelt (1945) clearly showed the structural basis for the control of the openings of the peritremes by the positional adjustment of the mandibular plate (stylophore), which in turn controls the area available for diffusion from the tracheal system. Oscillating movements of the mandibular plate, due to its structural relationship with the peritremes, could also result in active ventilation of the tracheal system.

Although references have been made to cuticular evaporation (Boudreaux 1958) in mites, the cuticle would be expected to be impermeable to water as is the case with other arthropods which have successfully invaded a terrestrial environment. Moreover a lipid layer has been demonstrated in the cuticle of this mite (Gibbs and Morrison 1959). In considering this aspect of the problem, the diapause form especially must be able to withstand severe water stress. The diapause females, a seasonal variation, are orange in color and the integument differs from that of the actively feeding form in that the striations appear to be solid in the former and broken in the latter (Pritchard and Baker 1952). Boudreaux (1956) showed the broken appearance of the striations on the summer form was due to semioblong lobes at the upper edge of the integumentary folds. Although referred to as the diapause form, it appears as active as the summer form at room temperature, except that it does not feed and no fecal material is visible in the midgut.

MATERIALS AND METHODS

The adult summer form of female *Tetranychus telarius* (Linnaeus) (=*T. urticae* Koch) mites from cultures reared on Fordhook 242 lima beans at 80°±3° F., 60% relative humidity, and 500 foot-candles continuous illumination, were used in this work. Diapausing females were picked out, as they appeared, from cultures of summer-form mites growing under greenhouse conditions (minimum night temperature 60° F.) during winter day length.

The mites were weighed on a quartz helix balance whose displacement was read with a cathetometer. This displacement was small enough to occur through an essentially linear range and the weight change of the mite was determined to four parts per hundred. The mite was weighed in an aluminum cage perforated to give about 50 percent open area. This cage was necessary to prevent the mite from crawling off the quartz fiber and to indicate if eggs or feces were deposited during the weighing period. The weighing chamber was controlled ±2° C. and various amounts of silica gel were used to adjust the relative humidity within ±5%. The mite remained on the balance throughout the period of weight determination except when removed for observation.

Summer forms and diapause forms were weighed without previous treatment to follow the normal weight loss. Summer forms were treated with three materials to isolate the factors involved in their water loss. Cyanide-killed mites were used to study losses from the tracheal system with a minimum disturbance of the cuticle; cyanide-killed mites were washed with cold chloroform to measure the increased water loss caused by disorganization of cuticular waxes and/or lipids; and live mites were dusted with amorphous silica gel (SG78 Davison Chemical C. Fl. type) and as much as possible of the material was immediately brushed off. This procedure was used to disorganize the cuticle without killing the mite and thus prevent free diffusion from the tracheal system.

The tracheal system was sealed to follow the water loss other than that resulting from diffusion through

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the peritremes. These measurements were made on 25 cyanide-killed mites. The anterodorsal surface of the mite was embedded in a minimum layer of petroleum jelly smeared on aluminum foil, and the weight measured. This treatment effectively sealed the peritreme plus the surrounding area of cuticle.

The water loss of the teliochrysalis was followed by weighing groups of ten in a small aluminum cage. Weighings were terminated when a sharp drop in weight occurred. A rapid change in weight was associated with an individual molting.

The behavioral response of the summer form of mites to high and low humidity was determined in the following manner. The change in the position of the mandibular plate was observed when the mite was exposed in turn to an airstream dried by passing through a dried cotton-packed adsorber and a silica gel adsorber and saturated by passing through the bubblers in series.

The average water content of mites was found by weighing to constant weight at 100° C.

The oxygen consumption of the mites was measured with a Misco differential type microrespirometer[2] held at 27°±0.1° C. in a water bath. The mites were collected in groups and divided into two samples. One sample was held in a saturated atmosphere and the other held over $CaCl_2$ for 20 hours prior to use. During the oxygen measurements, 10/100 W/V KOH was used with the mites from the wet atmosphere, and 135/100 W/V KOH was used with the mites from the dry atmosphere for CO_2 adsorption. These concentrations were chosen to give approximate relative humidities of 90% and 10% in the respirometer (Peterson 1944). The control mites and the diapauic mites were measured directly after collected.

The rate of water intake was measured by feeding a solution made up as follows: 0.015 M PO_4, 5% glucose, 2% amaranth red in 0.05 pH 7.2 tris buffer. This solution had an observed count from P^{32} of 1354±20 C.P.M. per 0.0025 μl. The dye was used to observe feeding activity. The mites were fed through a cast nylon membrane, Dupont Zytel No. 69, dissolved by heating in isobutyl alcohol (Boyd 1955) and cast in a warm-water bath. The membrane was supported on a thin layer of cork previously soaked in the feeding solutions.

After feeding, as indicated by the colored solution in the hindgut, single mites were rinsed in water and counted. The observed count was converted to a volume measurement. All counting was done with a thin-end window GM tube under constant geometry.

RESULTS

The weight losses of typical individual mites under nonfeeding conditions are shown in figs. 1 and 2. At the temperature range of 30° C. and below, the summer form can regulate its water loss down

[2] The respirometer was modified with a bolt in each corner of the cover.

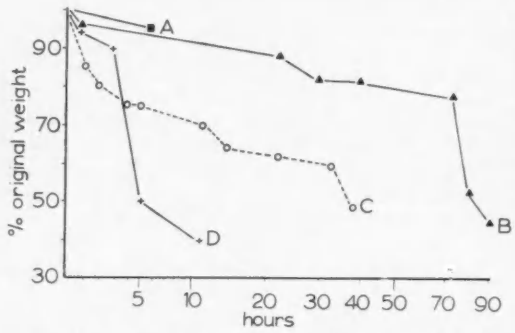

FIG. 1.—Weight loss of female mites at 30° C., 15%-20% relative humidity, except D at 50° C.: (A) teliochrysalis, (B) diapause form, (C,D) summer form, dead at final measurement.

to 15%-20% relative humidity for at least 24 hours (fig. 1,C). Its weight loss under the above conditions is characterized by an initial loss to 35% to 45% of the original weight followed by a stable period of up to 24 hours. Toward the end of this period of regulation, the mite becomes moribund and death occurs with a rapid loss of weight. In the temperature range above 45° C. there is a constant loss of weight without the stable period characteristic of lower temperature (fig. 1,D). At 45° C. the mites can survive up to 4 hours at 100% relative humidity, however at 50° C. a saturated atmosphere no longer shows a protective action.

At 30° C. and at relative humidities greater than 75%, the mites showed little if any weight loss in up to a 3-day period.

The teliochrysalis, up to the time of molting, showed no appreciable weight loss (fig. 1,A).

As shown in fig. 1,B, the diapause mite was highly resistant to desiccation. It was able to survive a greater percentage weight loss than the summer form, surviving greater than 50% weight loss.

Mites treated with cyanide, chloroform, and silica gel prior to weighing showed typical differences in

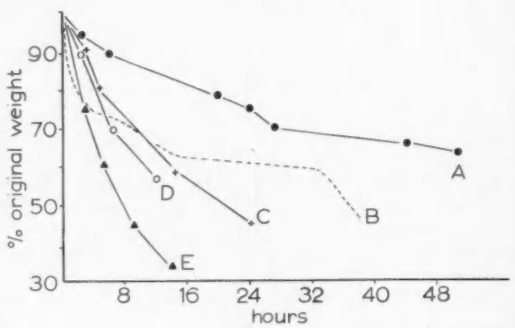

FIG. 2.—Weight loss at 30° C., 15%-20% relative humidity of treated female mites: (A) trachea sealed, (B) untreated, (C) SG78 silica gel-treated, (D) HCN-treated, (E) $CHCl_3$-treated.

weight loss (fig. 2). Mites killed with cyanide (fig. 2,D) showed a constant gradual loss which reflects diffusion from the tracheal system. The chloroform-treated mites (fig. 2,E) showed an increased loss over the cyanide-killed mites due to a change in the permeability in the cuticle. The silica gel-treated mites (fig. 2,C), although alive, were not able to arrest their water loss, and their weight decreased rapidly. However, silica gel-treated mites, when held in a saturated atmosphere, survived up to 4 days. The mites with the sealed tracheal system (fig. 2,A) showed less weight loss than the cyanide-killed mites; this weight loss may reflect that which occurs through the cuticle. The hibernating forms were so resistant to cyanide that it was not practical to study their weight loss by this method.

When the summer-form mites were observed under the saturated and dried airstream, the characteristic position of the mandibular plate was extended in wet air and retracted in dry air, indicating that the mites can adjust the rate of diffusion from the tracheal system. Three to four hours were required for the mite to adjust to the dry airstream. The characteristic position of the peritremes in the diapause form is completely withdrawn under the epistome.

The average water content of the summer form of mites was 70%, that of the diapause mites was 60%. The individual weights of the summer mites fell within the extremes of 17 to 22 μg., the range for the diapause mites was 10 to 15 μg. This difference in weight can be accounted for by not only the difference in water content but also the absence of fecal material in the midgut of the diapause forms.

The oxygen measurement of matched groups of mites is given in table 1. The holding period was chosen to place the mites in the stable period of the curve as shown in fig. 1,C. At this time the mites, under water stress, show an average of 70% reduction of oxygen consumption. The diapause forms, measured after removal from the plant, showed the lowest rate of respiration.

The mites, when fed on the red solution, bypassed the midgut and the liquid appeared directly in the hindgut as a thin red streak along the dorsal midline. The greater the intake, the farther the colored solution progressed posteriorly. After 1 hour, mites with a full hindgut were counted and were found to have taken in between 0.0013 μl and 0.0046 μl of solution. Several mites kept under continual observation while feeding, and counted as soon as the hindgut was filled, were found to have taken in a maximum of 0.0033 μl within 20 minutes.

Two mites, while on the planchet, passed urine containing amaranth, leaving the gut colorless. The first case had a count indicating an intake of 0.0039 μl with 5.8% of the count in the urine. The second case had a count indicating an intake of 0.0019 μl with 10% of the count in the urine. These results were checked by feeding previously labeled mites on distilled water until the hindgut was emptied of colored solution and finding the activity remaining in the mite.

DISCUSSION

The initial weight loss from the normal adult female mites and the cyanide-treated mites was similar enough to suggest that the major loss of water occurred from the tracheae. However, the weight-loss pattern of the live mite was characterized by a stable period of 20 hours duration, starting between 5 and 10 hours after the onset of the water stress. This stable period is also associated with a reduction of oxygen consumption as indicated in table 1.

Table 1.—Oxygen consumption in adult female mites[a].

	10^{-3} x mm^3 O_2/mite/hr.
Summer form	
control[b]	26.9 ± 0.8
low R.H.[c]	7.2 ± 0.7
high R. H.[d]	26.1 ± 2.5
Diapause form[b]	5.2 ± 0.5

[a] Measured in groups of 14-141, three replicates per measurement.
[b] Mites removed from plants immediately prior to measurement.
[c] Mites held over $CaCl_2$ 20 hours prior to measurement.
[d] Mites held over H_2O 20 hours prior to measurement.
All measurements at 27° C.

The time required to reach the stable period may reflect an adjustment made in the metabolic rate in order to reduce oxygen consumption so that the tracheae can be closed. Above 45° C. the mites were no longer able to control water loss.

The mites treated with chloroform showed an increased loss compared with the cyanide treatment; this difference reflects the loss caused by damage to the cuticle, possibly to the lipid components demonstrated by Gibbs and Morrison (1959).

The silica gel-treated mites, although possessing an intact tracheal system, showed a steady loss in weight with death resulting. The toxic effect was lost when the mites were held under saturated conditions. These results indicate that the toxicity of this silica gel resulted from physical damage to the cuticle and not from its fluoride content.

The water-loss rate from mites with sealed tracheae was similar to the rates from both the overwintering form and the summer form during the stable period. This decrease in water loss indicates that control of diffusion from the tracheal system is the most important variable factor in the mites' water balance at low relative humidities.

The response of the mandibular plate to changes in humidity indicates the mites are sensitive to a vapor pressure deficit and are able to exercise a degree of control over the rate of diffusion from the tracheae. This control of water-vapor loss from the tracheal system is limited by the metabolic rate of the mite. Under severe water stress the mite can reduce its oxygen consumption and seal off its tracheal system for longer periods of time. The

mite thus can conserve water both by its relatively impermeable cuticle and by controlling the rates of diffusion through its peritremes. This control over the peritremes would also provide for the regulated excretion of water at low humidity when actively feeding rather than by cuticular evaporation. The failure of mites to lose water at high relative humidities would indicate that the water in the diet must be eliminated in the urine when feeding under these conditions. As the initial slope of the water loss line at 30° C. and 15%-20% relative humidity shows the mite can lose as much as 10% of its initial weight per hour, a significant part of the water intake can be eliminated through the trachea. This factor would serve to relieve the excretory system of handling all the water in the diet and may be one of the reasons why spider mite infestations in the field are associated with hot, dry weather.

The greater ability of the diapause form to conserve water is not due to any radical changes from the summer form but is based on the same factors. Apparently the diapause form is able to exercise a greater degree of control over diffusion from its tracheal system as reflected by its resistance to desiccation, by its resistance to cyanide, and by its lowered respiratory rate. As the rate of weight loss in the diapause form was less compared to that of the summer form with sealed trachea, a decrease in water loss through the cuticle can also be postulated. This decrease may be due to the reduction in surface area of the diapause form, rather than any basic change in the cuticle. As no pore canals are present in this mite (Gibbs and Morrison 1959), no new materials can be deposited upon the surface. When the potential diapause forms emerge from the teliochrysalis stage the characteristic dorsal integumentary lobes are not present and this results in a decreased surface area (Boudreaux 1961). Intermediate forms of various degrees were found in the greenhouse cultures, which were green-orange in color and contained various amounts of the black fecal pellets. Upon isolation they where found to exibit a change to the typical orange color, void all their fecal pellets, and exibit resistance to cyanide. This color change has been described as due to a pigment in the blood (Ewing 1914, Cagle 1949). However, the pigment in whole mounts appeared to be concentrated in the midgut and frozen sections showed that the colored material was located mainly in the walls of the midgut. This colored material appeared to be droplets of a lipidlike substance which was soluble in chloroform and insoluble in water. This process indicates a possible role of fat storage in the diapause form. Fat metabolism by the diapause form would have the advantage of serving as a source of metabolic water.

A further factor in the ability of the overwintering form to withstand water stress is its ability to survive a greater percentage weight loss than the summer form. The individual from figure 1 survived a greater than 50% loss of initial weight.

The teliochrysalis, the last molting stage, showed no water loss in periods up to 6 hours. As this is a nonfeeding stage, conservation of water is required for survival. The formation of the new cuticle must interfere with diffusion from the tracheal system.

The explanation for the passage of colored liquid directly to the hindgut and bypassing the midgut demands that the end of the esophagus be in contact with the entrance to the hindgut. Blauvelt (1945, plate 11) showed that this is morphologically possible. The esophagus ends in a raised, funnel-shaped esophageal valve within the midgut which is larger in diameter than the opening to the hindgut. This esophageal valve could serve to make the necessary connection for a direct passage to the hindgut. Contraction of the dorsolongitudinal muscles would serve to lower the entrance to the hindgut down upon the esophageal valve. By this means particulate matter could enter the midgut and fluid passed to the hindgut, thus relieving the digestive area of the burden of handling a large volume of water. Blauvelt (1945) states that the food in the midgut appears as food balls plus recently acquired food material of chloroplasts and other material in a flocculent state, indicating injestion of particulate food into the midgut.

As shown by the removal of phosphate, but not amaranth, (M.W. 604.5) the hindgut can absorb ions and possibly other small molecules from the urine. Rodriguez (1954) has shown that female mites not only accumulate P^{32} but also use a large amount in egg formation.

The fluid intake of mites, which amounts to as much as 20% to 25% of the total weight per hour, required large amounts of urine to be passed when actively feeding. Under conditions of low humidity as much as one-half of this water excretion can be accounted for by diffusion from the tracheae.

The adult mite faces two extremes of water stress; the actively feeding summer form, under saturated conditions, must excrete large volumes of watery urine and avoid overloading its digestive system, while the nonfeeding diapause form must be able to prevent desiccation. Moreover, the same stage in its life cycle must be able to adapt to both extremes. Morphological as well as physiological factors have played a role in this extremely successful animal.

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The Nervous System of Certain Abdominal Segments and the Innervation of the Male Reproductive System and Genitalia of *Hyalophora cecropia* (Lepidoptera: Saturniidae)[1]

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ABSTRACT

The musculature and its innervations in the fourth pregenital abdominal segment of the adult male cecropia moth are described. Dorsal, ventral, and transverse pairs of nerve roots arising from the pregenital segmental abdominal ganglion seem homologous with those described for the pregenital segments of certain Orthoptera, the neuropteran *Chauliodes formosanus*, and the cecropia larva. In this lepidopteran, as in those other orders, the dorsal nerve fuses with the transverse nerve of the preceding ganglion, and the spiracular muscles are innervated from the fused branches of the dorsal and transverse nerves. The ventral nerve in this species and in some Orthoptera fuses with the transverse nerve of its own segmental ganglion. The homologies in such widely separated insect orders support the concept of a basic segmental nerve pattern within the Hexapoda. The terminal abdominal ganglion lies in the sixth abdominal segment in the cecropia male and gives rise to the lateral nerves of the sixth, seventh, and eighth abdominal segments, as well as the trunk giving rise to the branch nerves that innervate the reproductive system and the genital musculature. The vasa deferentia loop beneath the lateral nerves innervating the rectum, anal tube, and body wall in the anal area. If these relationships are homologous with the position of the lateral nerves of the 11th abdominal segment in Orthoptera, the nerves passing above the vasa deferentia in this moth are the lateral nerves of the 11th abdominal segment, which is no longer recognizable as a segment in *Hyalophora cecropia* (L.).

This study was undertaken to contribute to our knowledge of the abdominal musculature and its innervation in the Lepidoptea; an earlier publication (Libby 1959) discussed the musculature and its innervation in the cecropia larva.

The assumption that at some time in the past history of the Hexapoda the abdominal segments, as arthropod leg-bearing somites, had essentially the same structure as the primitive thoracic and gnathal segments has led to the concept of an underlying homology of segmental musculature. Previous work based upon this concept has supplied important evidence on the evolution of the insect thorax and appendages. Therefore, in a morphological study of the insect abdomen and its appendages, an attempt must be made to determine the presence of a basic plan of organization.

The concept of a basic plan of segmental musculature implies the presence of a basic primitive pattern of nerves to these serial muscles. If such a basic plan of homologous segmental musculature and innervation was present in each segment of this ancestor, the nerves innervating the segmental muscles of the insect would present a picture of the modifications of the basic primitive innervation pattern. Since the essential purpose of a nerve is to transmit an impulse, it would seem that selective pressures during the evolution from a primitive form to the present insect form would be of a lesser magnitude upon the nerves than upon the structures served by these nerves. A profound change in body structure might arise from a shift of the origin or attachment of a muscle group within the segment, while the corresponding change in the nerve branch innervating that muscle might result in only a slight shift in the position at which the nerve branch passes from its ganglionic "root" (Schmitt 1958).

Morphological studies concerning the Lepidoptera have unfortunately been confined mainly to the lepidopterous larvae, and very few studies of the internal morphology of adult Lepidoptera are recorded in the literature.

A few studies on other insect orders are of value in establishing a basic segmental nerve pattern among the Hexapoda. Maki (1936) conducted a thorough study of the alderfly *Chauliodes formosanus* Peterson. Nesbitt (1941) studied the nervous system of the Orthoptera and related orders, and (1958) the nervous system of *Grylloblatta campodeiformis* Walker. Schmitt (1954) studied the nervous system of the pregenital abdominal segments of some Orthoptera.

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MacGillivray (1923) published on the value of landmarks in insect morphology. He states as follows: "The use of landmarks makes for a continuous advancement, any other method makes for the accretion of facts without marked advancement." Certain "landmarks" are of value in the establishment of homologies in the abdomen. One important landmark in the study of the insect abdomen is the spiracles. The thoracic musculature of the winged imaginal stage of insects has resulted in shifts of position in the thoracic spiracles, but the abdominal spiracles are believed to have retained a more primitive position within their respective segments (Weber 1927). Therefore, it would seem that the musculature and its innervations serving the abdominal spiracles would retain comparative positions not far removed from that of the basic primitive segmental plan. Schmitt (1954) makes use of landmarks in his study of the nervous system of the pregenital abdominal segments of some Orthoptera.

Only two studies of a comparative morphological nature that deal specifically with the cecropia silkworm are to be found in the literature. The writer (1959) published on the nervous system of certain abdominal segments of the cecropia larva. He found that the ventral nerve fuses with the transverse nerve of its own ganglion, the dorsal nerve fuses with the transverse nerve of the preceding segmental ganglion, and the spiracular muscles are innervated from the connective between the dorsal and the transverse nerves, as in those Orthoptera studied by Schmitt (1954). These criteria of homology in such widely separated orders as Orthoptera and Lepidoptera support the concept of a basic segmental nerve plan within the Hexapoda. Beckel (1958) published on the morphology, histology, and physiology of the spiracular regulatory apparatus of *Hyalophora cecropia* (L.). Although Beckel figures portions of the musculature and nervous system of a typical abdominal segment of larva, pupa, and adult of cecropia, he limits his report of segmental nerves and musculature to these preliminary figures, for he was primarily concerned with the spiracular regulatory apparatus and not with the segmental nerve plan.

No studies dealing specifically with the innervation of the reproductive system of adult male Lepidoptera have been found in the literature. Ruckes (1919) studied the male genital system in certain Lepidoptera. He describes the parts of the adult male genitalia and also the innervation of the reproductive system and genital area in the early pupal stage of *Hyalophora cecropia*.

The writer concerns himself herein with the external genitalia of the male cecropia moth only from the standpoint of the innervation of the genitalia and the associated musculature.

METHODS OF NOMENCLATURE

The nomenclature of the body musculature of the male cecropia moth used in this study is based upon the general outline of body musculature set forth by Snodgrass (1931). This system provides sufficient flexibility for tentative designations of musculature encountered in cases where this musculature does not happen to fit exactly into the general outline. Some of the designations chosen might not be morphologically distinguishable but are used for convenience as explained in the results wherever necessary.

The nomenclature of the musculature associated with the genitalia follows that of Forbes (1939). Owing to the complexity of both the structure and nomenclature of the Lepidopterous genitalia, the terms presented by Snodgrass (1957) will be followed.

The nomenclature applied to the male reproductive system is rather general and has long been established and accepted. The terms used for the various parts of the male reproductive system in this study are from Snodgrass (1935).

The nomenclature of the nervous system follows that of Schmitt (1954) as previously used by the writer (1959). The large nerves or "nerve roots" leaving the segmental ganglion are named according to the general areas of the segment innervated by them. The nerve branches from these roots are indicated by the designations of the muscle groups which they innervate, or by their innervation of the body wall either directly or by means of the peripheral nerve net.

METHODS OF ILLUSTRATION

The illustrations of nerves and muscles contained in this paper are of two types. The first type might be termed "semi-perspective" in that an attempt has been made to present a reasonably true picture of the nerve-muscle relationships within the abdominal segments. The illustrations cannot be considered as completely realistic, since some of the lines of the muscle groups indicated are represented as straightened and the nerve branch terminations in their muscles are only approximately indicated. The second type of illustration is found in the diagrams of the nerves in which the spatial relationships of the nerves are indicated but in which lettered designations are substituted for the muscles innervated or the integument. This type of diagram was employed since we are not concerned herein with the physiology of nerve endings but rather with the morphology of the nerve pattern as a guide in the establishment of homologies.

Points at which nerves pass but are not joined are represented by an interruption of the more laterad nerve, while nerve junctions are represented by the intersection of uninterrupted lines. Nerve branches passing beneath muscle groups are represented by broken lines.

RESULTS AND DISCUSSION

I. GENERAL.—The motor nerves and musculature of the fourth abdominal segment were studied by dissections of the male moths. It was found that by proceeding from the interior muscle groups to the

exterior muscle groups upon the body wall, the study of the muscles and their innervations could be most readily facilitated. The musculature was designated according to the general outline of body musculature presented by Snodgrass (1931).

The motor nerves were followed from their "roots" arising from the segmental ganglion to their terminations in muscles or upon the integument (figs. 1, 2).

The ventral nervous system consists of the postoral series of segmental ganglia and their connectives, constituting the ventral nerve cord (Snodgrass 1935). This study was concerned with the portion of the ventral nerve cord and its lateral nerve roots within the fourth abdominal segment, as a typical pregenital abdominal segment, and with the terminal ganglion and its lateral nerve roots. Each segmental ganglion is an oval mass of nerve tissue, continuous anteriorly and posteriorly with the interganglionic connectives. The interganglionic connectives of the cecropia male moth are fused as they leave the ganglion posteriorly and as they connect with the posterior segmental ganglion. Two lateral nerves and a median nerve arise from each segmental ganglion of the cecropia male moth. The paired anterior lateral nerves arising from the segmental ganglion are designated the dorsal nerves (DNv). The paired posterior lateral nerves arising directly from the ganglion are designated the ventral nerves (VNv). A median nerve (MdNv) arises from the segmental ganglion and passes posteriorly between the fused interganglionic connectives. The median nerve gives rise to the transverse nerves (TNv) which pass laterally from the median nerve just anterior to the posterior segmental ganglion. The segmental ganglion and its lateral nerves are covered with a continuous nucleated sheath, the neurilemma. In the male cecropia moth, the transverse ventral musculature (tv) or ventral diaphragm arises on the intersegmental folds and inserts on the mid-line of the ventral nerve cord (fig. 1). This attachment of the ventral diaphragm to the ventral nerve cord was previously reported by Beckel (1958). The attachment of musculature to the ventral nerve cord is, in any case, uncommon.

II. THE DORSAL NERVE.—The anterior nerve root from each of the abdominal segmental ganglion is herein designated the dorsal nerve (figs. 1, 2: DNv). The dorsal nerve leaves the segmental ganglion, passes beneath the transverse ventral musculature (tv) or ventral diaphragm and above the ventral internal medial longitudinal muscle groups (vim 1 to 5). The dorsal nerve passes dorsally beneath the ventral internal lateral muscle group (vil).

The first branch to leave the dorsal nerve arises between the ventral internal muscle groups (vim 1 to 5) and the ventral internal lateral muscle group (vil), passing posterodorsally. This first posterior branch of the dorsal nerve branches when it reaches the ventral internal lateral (vil) muscle group. One of these branches passes posteriorly beneath the ventral internal lateral (vil) muscle group innervating the body wall (bw) and the fine musculature which lines the body wall from the midventral line dorsad to just beyond the spiracle, designated the lateral tergosternal musculature (lts). The other of these branches passes above the ventral internal lateral muscle group (vil) innervating this muscle group. A fine branch of the first branch of the dorsal nerve arises from the dorsal nerve just beyond the origin of the first branch of the dorsal nerve. This fine branch passes ventrally and innervates the ventral internal medial muscle groups (vim 1 to 5).

A second branch arises anterodorsally from the lateral muscle group (vil). This second branch of the dorsal nerve passes beneath the ventral internal lateral muscle group (vil), as does the dorsal nerve. The second branch of the dorsal nerve then forks. One of the branches of the fork innervates the ventral scolopophorous organ (sco 1) and the other moves dorsally where it joins a branch of the transverse nerve (TNv) of the median system ventral to the spiracle at point "B" (fig. 6). The nerve branches, composed of the nerve fibers of both the second branch of the dorsal nerve and the connective from the transverse nerve, point "C" (fig. 6), innervate the occlusor of the spiracle (osp) and the lateral tergosternal musculature (lts) anterior to the spiracle.

A short branch runs from the dorsal nerve, point "A" (fig. 6), fusing with the transverse nerve of the median system where these nerves pass just posterior to the spiracle. A fine branch from the dorsal nerve passes beneath the transverse nerve and forks to innervate the lateral scolopophorous organ (fig. 2: sco 2) and the body wall (bw) just dorsad to the spiracle. The dilator or elastic opener of the spiracle is not composed of muscle tissue and receives no apparent innervation of its own (Beckel 1958).

The dorsal nerve continues to pass dorsally in the segment, passing above the dorsal internal lateral muscle group (dil). A branch of the dorsal nerve passes beneath the dorsal internal lateral muscle group innervating this muscle group.

A third branch leaves the dorsal nerve just dorsad to the dorsal internal lateral muscle group (dil). The third branch of the dorsal nerve moves dorsally and posteriorly in the dorsum of the segment, passing beneath the dorsal scolopophorous organ (sco 3). The third branch of the dorsal nerve gives rise to nerve branches which innervate the dorsal scolopophorous organ (sco 3) and the dorsal external muscle groups (de 1 to 5). This nerve branch passes beneath the dorsal internal medial muscle group (dim 1 to 3).

The main dorsal nerve passes above the dorsal scolopophorous organ (sco 3) and continues dorsally. A dorsal anterior branch passes from the dorsal nerve and forks to innervate the body wall of the tergum. The dorsal nerve then passes beneath the dorsal internal medial muscles (dim to 3) and a

branch of the dorsal nerve innervates these muscle groups. Next the dorsal nerve passes above the dorsal internal musculature (di), innervates these muscles, and appears to terminate in the body wall beneath the dorsal vessel.

The structures of the fourth abdominal segment of the male cecropia moth innervated by the dorsal nerve can be viewed in figures 1 and 2.

III. THE VENTRAL NERVE.—The posterior nerve root from each of the abdominal segmental ganglion is herein designated the ventral nerve (figs. 1, 2: VNv). The ventral nerve leaves the ganglion and passes obliquely posteriorly beneath the ventral internal medial muscle groups (vim 1 to 5).

The ventral nerve forks just beyond the ventral internal medial muscle groups (vim 1 to 5). The anterior branch of this fork gives rise to a nerve branch which passes beneath the ventral nerve and innervates lateral tergosternal musculature (lts) in the sternal region. Other branches arise from the anterior branch of the ventral nerve; they connect with the peripheral nerve net and innervate the fine lateral tergosternal musculature (lts) in the sternopleural area of the segment. The anterior branch of the ventral nerve also innervates the intersegmental fold (ISF) of the body wall at the insertion of the transverse ventral musculature or ventral diaphragm.

The posterior branch of the fork of the ventral nerve gives rise to a branch which innervates the ventral external muscle groups (ve 1 to 6). The posterior branch of the ventral nerve then passes posterodorsally and gives rise to the connective between the ventral nerve and the transverse nerve of

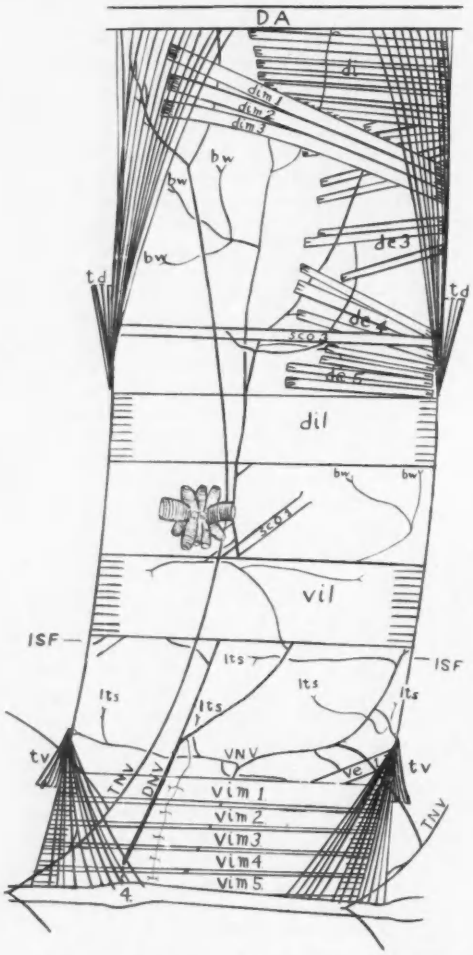


FIG. 1.—Nerves and muscles of the right side of the fourth abdominal segment of the male cecropia moth. First stage of dissection.

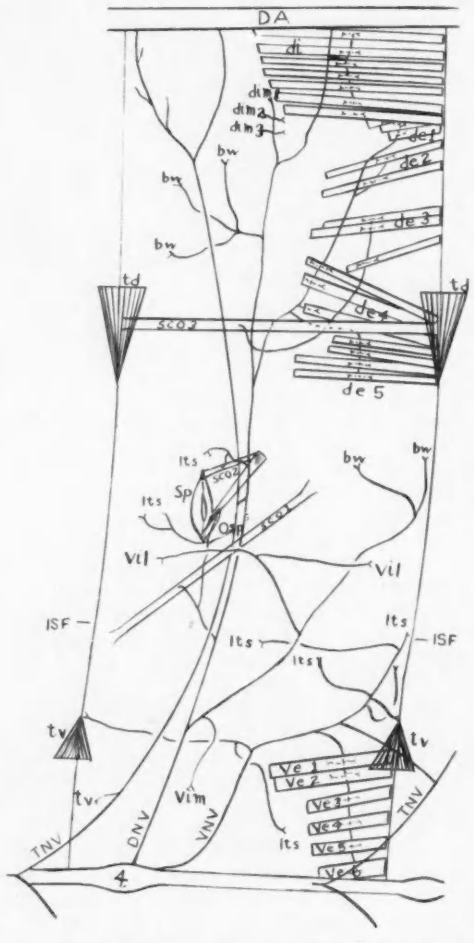


FIG. 2.—Nerves and muscles of the right side of the fourth abdominal segment of the male cecropia moth. Second stage of dissection.

the segment, which passes above the transverse ventral musculature or ventral diaphragm to join the transverse nerve at "B'". The posterior branch of the ventral nerve continues to pass posterodorsally and gives rise to a fine branch which innervates the posterior intersegmental fold (ISF) at the insertion of the transverse ventral musculature or ventral diaphragm.

The posterior branch of the ventral nerve continues to pass dorsally and gives rise to several fine branches which connect with the peripheral nerve net, innervating the fine lateral tergosternal musculature of the sternopleural region of the segment.

IV. THE TRANSVERSE NERVE.—The very short, unpaired, median nerve of a typical abdominal segment of the male cecropia moth arises as a separate structure from the fused intersegmental ganglionic connectives just anterior to the intersegmental line. The unpaired median nerve gives rise to a pair of transverse nerves.

The transverse nerves (fig. 1: TNv) pass above the fine transverse musculature or ventral diaphragm muscles which arise on the intersegmental fold of the body wall and insert on the ventral nerve cord. A fine branch of the transverse nerve passes to and innervates the transverse ventral musculature (fig. 1, 2: tv).

The transverse nerve continues to pass dorsally in the segment (fig. 1), passing mediad of the ventral internal medial muscles (vim 1 to 5) and the ventral internal lateral (vil) muscle group. Just dorsad of the ventral internal lateral muscle group (vil) the transverse nerve, arising from the abdominal segmental ganglion of the anterior abdominal segment, is joined by the second branch of the dorsal nerve of the abdominal segment. This "landmark" is designated point "B" (fig. 6). A nerve branch arising from this junction at point "C" (fig. 6), innervates the occlusor muscle of the spiracle (osp) and lateral tergosternal musclature anterior to the spiracle (fig. 2), as were previously stated in the discussion of the dorsal nerve. The transverse nerve is joined at point "B" by a second branch of the dorsal nerve arising at point "A" (fig. 6). No nerve branches emanate from this junction of the dorsal and transverse nerves. The transverse nerve passes obliquely anterodorsally from this junction, above the dorsal internal lateral muscle group (dil and the dorsal scolopophorous organ (sco 3). The transverse nerve forks in the dorsum of the segment. The posterior dorsal branch of the transverse nerve passes beneath the dorsal internal medial muscle groups (dim 1 to 3) and appears to terminate in the dorsal vessel. The anterior dorsal branch of the transverse nerve passes among the fine muscle fibers of the transverse dorsal (td) or alary musculature, innervating this musculature on both sides of the intersegmental fold (fig. 1). This was previously reported by the writer (1959) in his study of the cecropia larva. In this earlier study, the writer also referred to the study of the nervous system of the silkworm, *Bombyx mori* (L.) by Kuwana (1932). Kuwana showed that the transverse nerve does not terminate with the branches which innervate the transdorsal alary muscles, but also pass along the transverse dorsal alary muscles to connect with the heart nerve or lateral nerve of the dorsal vessel.

V. CRITERIA OF HOMOLOGY OF NERVES OF THE PREGENITAL ABDOMINAL SEGMENT.—To determine a basic segmental nerve plan requires the establishment of criteria of homology of nerves, and various "landmarks" within the pregenital abdominal segments are utilized in the establishment of criteria of homology. The dorsal and the ventral longitudinal muscles are considered primitive muscle groups of the segmental musculature, phylogenetically antedating the insects themselves. The abdominal spiracles are believed to be more primitive than the thoracic spiracles. Therefore, it would seem that the innervations of these primitive segmental structures should be homologous among different insect orders.

Schmitt (1954) used these segmental features to establish criteria of homology of nerves in his study of the nervous system of the pregenital abdominal segments of some Orthoptera, and the present study was initiated to determine if the same criteria of homology of nerves of the pregenital abdominal segments could be applied to another order of insects, the Lepidoptera, phylogenetically well removed from the Orthoptera. The nerve plan of a pregenital abdominal segment of *Acheta assimilis* F. as determined by Schmitt (1954) is presented in figure 4. The nerve plan of a pregenital abdominal segment of the adult of a neuropterous insect, *Chauliodes formosanus*, as determined by Maki (1936), is presented in figure 3. The nerve plan of a mid-abdominal segment of the *Hyalophora cecropia* larva, as determined by the writer (1959) is presented in figure 5. The nerve plan of the fourth abdominal segment of the cecropia male moth is presented in figure 6.

The Orthoptera are considered to be a primitive order of hemimetabola, the Neuroptera are considered to be one of the most primitive groups of holometabolous insects, and the Lepidoptera are counted among the higher orders of holometabolous insects.

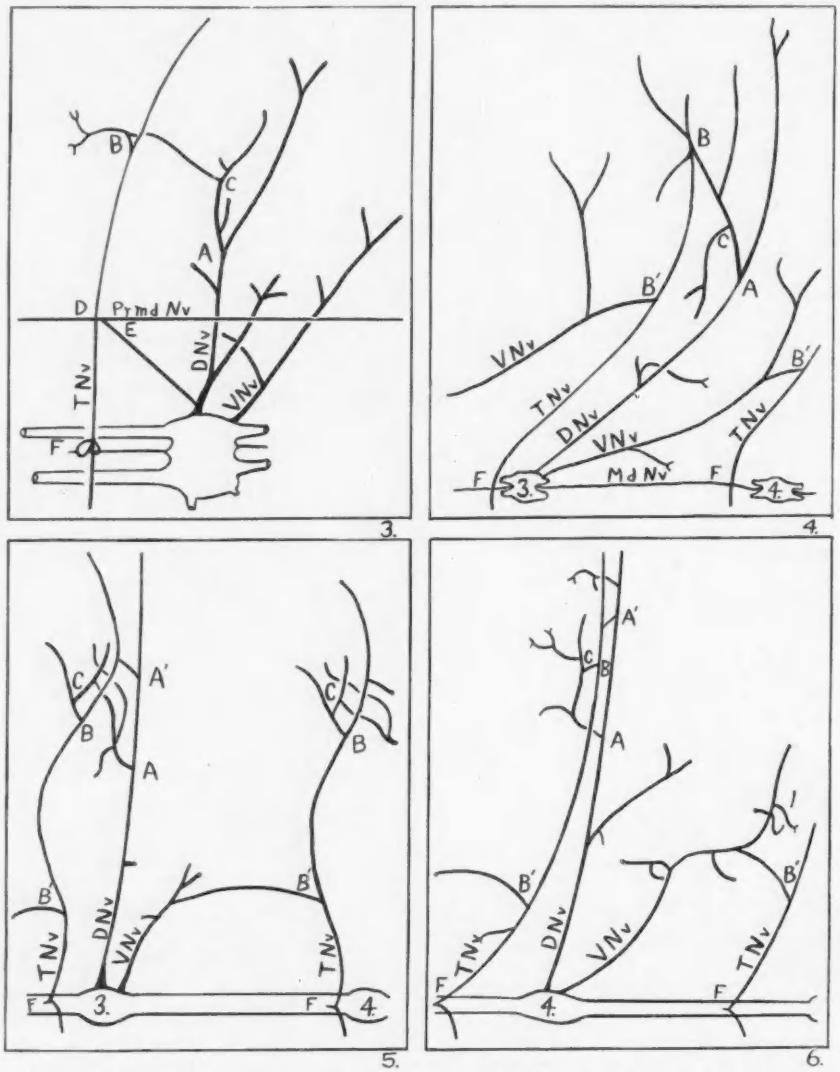
Various points used as criteria of homology can be established in the pregenital abdominal segmental nerve plans among these widely separated orders. In each of these pregenital abdominal segmental nerve plans, the spiracular musculature is innervated by a nerve branch that arises from the connective between the dorsal nerve (DN) and the transverse nerve (TNv), which has its origin in the preceding segmental ganglion. The points at which the connective nerve joins the dorsal nerve and the transverse nerve are designated respectively "A" and "B". The point at which the nerve branch innervating the spiracular musculature leaves the connective branch is designated "C" (figs. 3, 4, 5, 6).

The transverse nerve (TNv) joins the ventral nerve (VNv) at "B'" (figs. 4, 5, 6). This point of

homology is indicated by Schmitt (1954) as being present among some Orthoptera, and was found to be present in the cecropia larva (Libby 1959). Maki (1936) does not indicate such a connection of the transverse nerve (TNv) with the ventral nerve (VNv) in *Chauliodes formosanus* (fig. 3).

The establishment of these criteria of homology among such widely separated orders as the Orthoptera, Neuroptera, and Lepidoptera lends strong support to the concept of a basic segmental nerve plan among the Hexapoda.

VI. THE TERMINAL ABDOMINAL GANGLION AND THE INNERVATION OF THE REPRODUCTIVE SYSTEM AND GENITALIA.—The terminal abdominal ganglion of the male cecropia moth is found in the sixth abdominal segment. This ganglion gives rise to the lateral nerves of the sixth, seventh, and eighth abdominal segments and also innervates the reproduc-



FIGS. 3, 4, 5, and 6.—Diagrams of the nerve patterns of the right side of typical pregenital abdominal segments with points of homology indicated, viewed mesally. FIG. 3.—The neuropteran *Chauliodes formosanus* Peterson (after Maki 1936). FIG. 4.—The orthopteran *Acheta assimilis* F. (after Schmitt 1954). FIG. 5.—The lepidopteran *Hyalophora cecropia* (L.) (after Libby 1959). Larva. FIG. 6.—The lepidopteran *Hyalophora cecropia* (L.). Adult male.

tive system and the genital musculature as well as the body wall areas of the genital segments (figs. 7, 8).

The lateral nerves of the sixth and seventh abdominal segments (fig. 7: A, B) are serially homologous with the lateral nerves of the fourth abdominal segment, which was studied as a typical pregenital abdominal segment of the cecropia male moth (figs. 1, 2). The lateral nerves of the eighth abdominal segment are quite similar to those of abdominal segments 6 and 7 with some modifications (fig. 7).

The transverse nerves of the eighth abdominal segment arise from the median nerve, which extends caudad from the terminal abdominal ganglion (TG). The transverse nerve serving the right side of the eighth abdominal segment has a connection with the ventral nerve of the seventh abdominal segment. The transverse nerve continues to pass dorsally in the eighth abdominal segment and joins the dorsal nerve of the eighth abdominal segment. The transverse and dorsal nerves anastomose and pass dorsally together for a short distance. A branch arises from the fused transverse and dorsal nerves which innervates the vestigial eighth abdominal spiracle (VS) and the lateral tergosternal musculature (lts) in the area of the vestigial eighth abdominal spiracle.

The fused nerves then separate. Since one nerve branch passes anterodorsally to innervate the transverse dorsal (td) or alary muscles supporting the dorsal aorta, it is probably the transverse nerve (fig. 7: TNv8).

The dorsal nerve of the eighth abdominal segment passes caudad from the terminal abdominal ganglion (TG), moves obliquely posterodorsally across the seventh abdominal segment, and passes dorsally in the eighth abdominal segment. A fine branch of the dorsal nerve passes ventrally to innervate the ventral internal medial muscles (vim) which arise on the intersegmental fold between the seventh and eighth abdominal segments and insert on the heavily sclerotized intersegmental ridge between the eighth and ninth abdominal segments (SR). The dorsal nerve then gives rise to a branch which innervates the ventral internal lateral muscles (vil). The dorsal nerve of the eighth abdominal segment continues to pass dorsally, joining the transverse nerve for a short distance. A branch arises from the dorsal and transverse nerves which innervates the vestigial spiracle (VS) of the eighth abdominal segment and

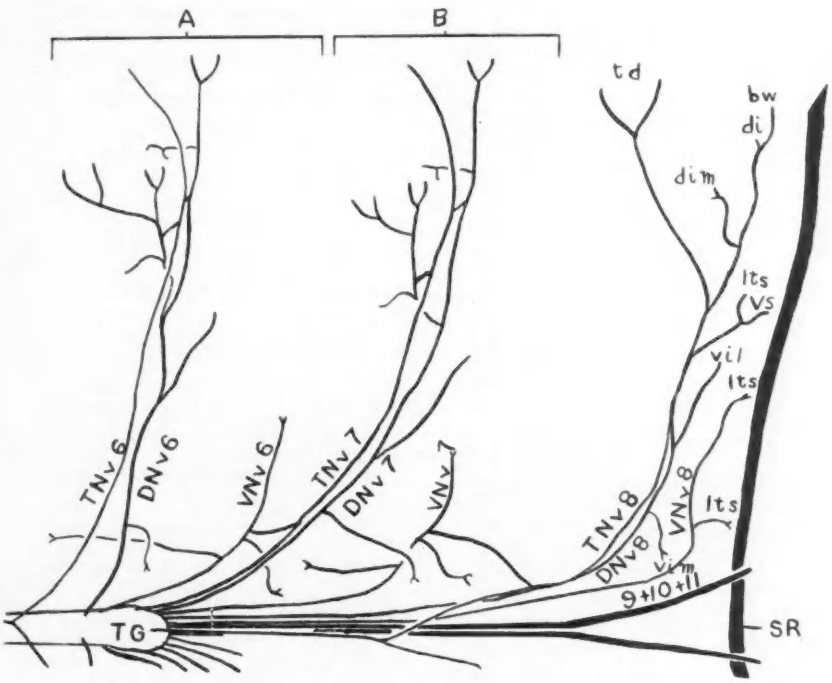


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FIG. 7.—Diagram of the terminal abdominal ganglion of the male cecropia moth and its lateral nerves. Right side, viewed mesally.

lateral tergosternal musculature (lts) in the area of the vestigial spiracle, as previously stated. The fused nerves then separate. Since a nerve branch passes dorsally to innervate the dorsal internal medial (dim) and the dorsal internal (di) muscle groups, it is probably the dorsal nerve (fig. 7:

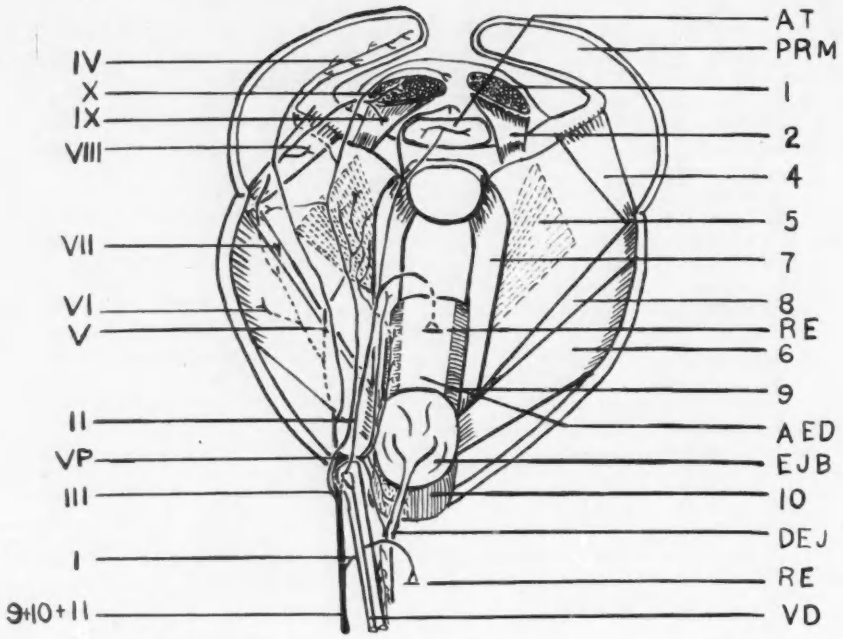

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FIG. 8.—The structures of the genital region of the male cecropia moth and their innervations, viewed dorso-ventrally.

ABBREVIATIONS USED ON THE FIGURES

A to F	upper case letters indicate points used as criteria of homology
AED	aedeagus
AT	anal tube
bw	body wall
DA	dorsal vessel
de 1 to 5	dorsal external muscles
DEJ	ductus ejaculatorious
di	dorsal internal muscles
dil 1 to 3	dorsal internal lateral muscles
dim	dorsal internal medial muscles
DNv	dorsal nerve
EJB	ejaculatory bulb
ISF	intersegmental fold
lts	lateral tergo-sternal muscles
MdNv	median nerve
osp	occlusor muscle of the spiracle
PRM	paramere
PrmdNv	paramedian nerve
RE	rectum
sco 1 to 3	scolopophorous organs
Sp	spiracle
SR	sclerotized ridge between abdominal segments eight and nine
TG	terminal ganglion
TNv	transverse nerve
VD	vasa deferentia
ve 1 to 5	ventral external muscles
vil	ventral internal lateral muscles
vim 1 to 5	ventral internal medial muscles
VNv	ventral nerve
VP	vesicular plexus
1	depressor muscles of the uncus
2	retractor muscles of the anal tube
4	extensor muscles of the valve or paramere
5	flexor muscles of the claspers or parameres
6	protractor muscles of the aedeagus
7	retractor muscles of the aedeagus
8	flexor muscles of the valve or paramere
9	muscles ensheathing the base of the aedeagus
10	muscles arising on the ejaculatory bulb and inserting on the aedeagus
9+10+11	nerve trunk of the terminal abdominal ganglion which innervates the genitalia and associated musculature
I to X	Roman numerals indicate branches of nerve trunk 9+10+11.

DNv8). The dorsal internal medial (dim) and dorsal internal (di) muscle groups are well developed in the eighth abdominal segment, having their attachments on the intersegmental fold between the seventh and eighth abdominal segments and the sclerotized ridge between the eighth and ninth abdominal segments.

The ventral nerve of the eighth abdominal segment passes caudad from the terminal abdominal ganglion (TG) to innervate the lateral tergosternal musculature (lts) in the ventral-pleural areas (fig. 7). Unlike the ventral nerves of other pregenital abdominal segments, the ventral nerve of the eighth abdominal segment (VNv8) has no connections with other nerves.

The lateral nerves of the genital segments leave the terminal ganglion as paired heavy nerve trunks composed of the lateral nerves of the ninth, tenth, and eleventh primitive abdominal segmental ganglia. This nerve trunk, designated 9+10+11, passes caudad from the terminal ganglion (TG) and passes above the sclerotized ridge (SR) between the eighth and ninth abdominal segments (fig. 7).

The first branch (I) from nerve trunk 9+10+11 (fig. 8) leaves the nerve trunk and passes dorsally through the loop of the vas deferens (fig. 8: VD) to innervate the rectum (RE). The nerve trunk 9+10+11 continues to pass caudad, and a second branch (II) arises from it which also passes through the loop of the vas deferens. Nerve branch II passes posteriorly, giving rise to a fine branch which passes dorsally to innervate the rectum. Nerve branch II continues to pass posteriorly to innervate the anal tube (AT) and the body wall in the region of the anus (fig. 8).

Snodgrass (1936) studied the anatomical relations between the vas deferentia and the posterior segmental nerve trunks of the abdomen in Orthoptera. His study showed that the male genital ducts must originally have crossed over the lateral nerves of the tenth abdominal segment and attached to the ectoderm behind these nerves. The union of the vasa deferentia with the median ductus ejaculatorius, however, necessitated a median and forward migration of the posterior ends of the lateral ducts. The correlated forward retraction of the posterior part of the ventral nerve cord then drew the large nerves of the cerci over the incurved ends of the vasa deferentia. Therefore, in the definitive condition, the vasa deferentia are always looped *beneath* the cercal nerves or lateral nerves of the eleventh segment, but they lie *dorsal* to the other lateral nerve trunks of the abdomen. Snodgrass states that this condition could follow only from one in which the primitive genital ducts turned downward to the body wall between the nerve trunks of the tenth and eleventh abdominal segments.

The distinct morphological identities of the ninth, tenth, and eleventh abdominal segments are lost in the male cecropia moth. Nerve branches I and II (fig. 8), as described above, arise from nerve trunk 9+10+11 and pass through the loop of the vasa deferentia, indicating that these branches are probably the lateral nerves of the primitive eleventh abdominal ganglion. It should be noted that these nerve branches innervate the rectum, the anal tube, and the body wall in the anal area. These portions of the proctodaeum are formed in embryogeny by an ectodermal invagination of the posterior terminal region of the embryo. Contemporary embryological theory holds that the eleventh abdominal segment is the most posterior primitive abdominal segment possessing a segmental ganglion.

The third branch (III) from nerve trunk 9+10+11 (fig. 8) arises from the nerve trunk in the same area as does nerve branch II. Nerve branch III passes posteriorly beneath the vasa deferentia and branches to innervate the body wall at the articulation of the paramere (PRM) and terminates on the body wall between the origins of the paired muscle group 1 (fig. 8), the depressors of the uncus.

Just posterior to the third branch of the trunk there is a nerve plexus called the vesicular plexus (VP). This plexus gives rise to three nerve branches: an anterior branch which innervates the vasa deferentia (VD) and the ductus ejaculatorious (DEJ). Two posterior branches arise from the vesicular plexus, the first of which passes posteriorly and innervates muscle group 7, the retractors of the aedeagus, while the second innervates muscle group 9, which is a group of fine muscle fibers ensheathing the base of the aedeagus. The second posterior nerve branch of the vesicular plexus (VP) then passes posteriorly to innervate muscle group 5, the flexors of the claspers or parameres (PRM). A fine branch of the second posterior nerve branch passes anteriorly from this nerve branch near the vesicular plexus (VP) and innervates muscle group 10, which consists of fine muscle fibers arising on the base of the ejaculatory bulb (EJB) and inserting on the base of the aedeagus (AED), ventral to the ejaculatory bulb (fig. 8: EJB).

Nerve trunk 9+10+11 continues to pass posteriorly. Nerve branch IV arises from the nerve trunk just anterior to muscle group 6, the protractors of the aedeagus. Nerve branch IV passes above muscle group 6, moving obliquely and posteriorly above muscle group 4, the extensors of the valve or paramere (PRM), entering the paramere where it innervates the body wall (fig. 8).

Nerve trunk 9+10+11 passes posteriorly beneath muscle group 6, the protractors of the aedeagus, where nerve branch V arises from the nerve trunk and passes posteroventrally beneath muscle group 6 and above muscle group 8, the flexers of the valve or paramere (PRM), to innervate muscle group 5, the flexors of the clasper or paramere (PRM). The next branch to arise from nerve trunk 9+10+11 is nerve branch VI which innervates muscle group 6, the protractors of the aedeagus (fig. 8).

Nerve trunk 9+10+11 continues to pass posteriorly innervating the musculature associated with the

genitalia. This nerve trunk passes above muscle group 8, the flexors of the valve or paramere (PRM), giving rise to nerve branch VII, which innervates muscle group 8. Nerve trunk 9+10+11 then passes above muscle group 4, the extensors of the valve or paramere (PRM) where nerve branch VIII leaves the nerve trunk to innervate muscle group 4. The nerve trunk continues to pass posteriorly and forks, giving rise to nerve branches IX and X which terminate in muscle groups 2, the retractors of the anal tube, and 1, the depressor of the uncus, respectively (fig. 8).

As stated in "Methods of Nomenclature", the nomenclature of the musculature associated with the genitalia follows that of Forbes (1939). In his study of the genital musculature of Lepidoptera Forbes indicates the presence of a slender muscle designated 3 which he feels is morphologically an extensor of the valve or paramere. Muscle group 3 was not found by the writer in the cecropia male moth, where the massive muscle group 4 (Forbes 1939) acts as the extensor of the valve or paramere.

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Rotation of Male Terminalia in *Aedes taeniorhynchus* (Diptera: Culicidae) as Affected by Temperature[1]

MAURICE W. PROVOST, PATRICK T. M. LUM, AND NINA BRANCH

ABSTRACT

Hourly samples of 20 male *Aedes taeniorhynchus* each were examined for degree of rotation of terminalia up to 48 hours after emergence at 18°, 22°, 25°, 30°, 32°, and 36°. Terminalia were ascribed to Stage I if unrotated, Stage II if rotated up to 135°, and Stage III if rotated 135° to 180°. Rotation frequently stopped between 135° and 180°. Rate of turning was directly related to temperature up to 135°; unknown factors entered the scene beyond 135°. Induced copulation was possible with 90° of rotation. Stage III was considered terminalial maturity. At the six given temperatures, terminalial maturity was reached by 50% of mosquitoes in, respectively, 38, 23, 20, 11, 15, and 12 hours. At the last five temperatures, 95% of mosquitoes reached maturity at 42, 33, 30, 27, and 30 hours. The curves for the relationship of temperature to terminalial rotation and to pupal duration in this mosquito are almost identical, 3 hours of pupal duration being equivalent at the same temperature to 1 hour of sexual maturation. The use of terminalial rotation as an adult aging technique is discussed and exemplified.

Rotation of male terminalia in the southern saltmarsh mosquito, *Aedes taeniorhynchus* (Wiedemann) was used in 1952 by E. T. Nielsen (1958) to determine the adult age in field-caught insects. Since that time he and others of the staff at this research center have employed the technique very successfully with this and other mosquitoes. The technique has proved essential to studies of the migratory exodus. Dr. Nielsen early recognized that temperature affected the rate of rotation and performed preliminary measurements of that effect. The present paper reports a more detailed study of the effect of temperatures between 18° and 36° C. on the rotation of male terminalia in *Aedes taeniorhynchus*.

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METHODS

Pupae of *A. taeniorhynchus* were kept in shallow pans and observed until the emergence of adult males occurred in appreciable numbers. The objective then became the segregation of 20 males emerged within the same 5 minutes, placing them in a large test tube with sugar water available, and moving them at once to a bioclimatic room of a given temperature. A complete test at one temperature required 48 such groups of 20 since the groups were progressively killed (with chloroform) at 1-hour intervals in order to give an hourly picture of the rotational progress over a 2-day period. The samples were kept in 70% alcohol until they could be examined for rotation of terminalia, which was usually within a week. The following divisional markers were used to categorize five classes of the rotation of male terminalia (fig. 1):

Class 1. 0 degrees. No sign of rotation of segments posterior to the seventh segment. When the abdomen is viewed laterally, the pleural area is a fairly precise continuous line, one basistyle lies exactly behind the other, both appearing as one in profile, and the dististyles curve dorsally.

Class 2. 0 plus to 90 degrees. When the abdomen is viewed laterally both basistyles can be seen and the pleural line is no longer straight but jogged between the seventh and eighth segments. This class reaches its rotational limit when a precise ventral view reveals one basistyle to lie directly behind the other in perfect profile. This view will also show easily when the rotation has advanced beyond 90 degrees, however slightly.

Class 3. 90 plus to 135 degrees minus. When the abdomen is viewed laterally the dististyles curve ventrally and both can be clearly seen.

Class 4. 135 plus to 180 degrees minus. From a lateral view the basistyles can both still be seen although one will almost cover the other, and the pleural area again appears to be approaching an unbroken line.

Class 5. 180 degrees. The dististyles curve ventrally, the basistyles appear laterally in perfect profile so that they seem as one, and the pleural line is continuous and unjogged on one side of the abdomen. The other side is jogged between the seventh and eighth segments, apparently because of some alteration or shrinkage in the terminal segments during rotation.

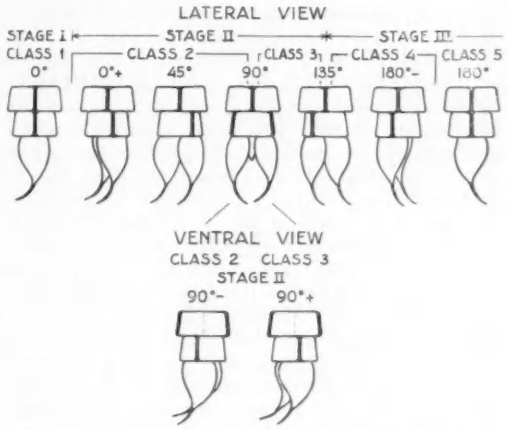

FIG. 1.—Appearance of male terminalia in *Aedes taeniorhynchus* in proceeding from 0° to 180° rotation. See description in text.

RESULTS

Male *A. taeniorhynchus* can be made to copulate when the terminalia have rotated 90° or more by using the McDaniel-Horsfall (1957) technique. This is important to an understanding of the results of experiments with rotation of the terminalia.

Table 1.—Rate of terminalia rotation in *A. taeniorhynchus* males at six temperatures. Computed as 5-hour running average percentages every 3 hours.

Age (Hours)	Percent in each of three stages																	
	18°			22°			25°			30°			32°			36°		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
3	100	—	—	95	5	—	90	10	—	77	23	—	74	26	—	67	33	—
6	100	—	—	91	9	—	42	58	—	34	59	7	21	77	2	18	78	4
9	91	9	—	70	30	—	5	95	—	16	60	24	4	74	22	3	72	25
12	74	26	—	35	65	—	—	93	7	3	31	66	—	60	40	—	49	51
15	59	41	—	11	84	5	—	82	18	1	10	89	—	61	39	—	29	71
18	26	74	—	2	84	14	—	72	28	—	13	87	—	35	65	—	18	82
21	9	91	—	—	68	32	—	39	61	—	6	94	—	16	84	—	11	89
24	8	92	—	—	43	57	—	21	79	—	4	96	—	8	92	—	13	87
27	—	99	1	—	30	70	—	18	82	—	2	98	—	5	95	—	7	93
30	—	87	13	—	20	80	—	10	90	—	—	100	—	7	93	—	3	97
33	—	65	35	—	16	84	—	4	96	—	—	100	—	7	93	—	6	94
36	—	58	42	—	10	90	—	5	95	—	—	100	—	4	96	—	6	94
39	—	51	49	—	9	91	—	4	96	—	—	—	—	3	97	—	1	99
42	—	31	69	—	5	95	—	9	91	—	—	—	—	3	97	—	1	99
45	—	27	73	—	4	96	—	7	93	—	—	—	—	7	93	—	8	92

Class 5 rotation (= 180°) was not always reached in these tests, the rotation ending between 135° and 180° in the majority of specimens. A few stopped between 90° and 135° even at the highest temperatures. The time required to attain 135° was temperature related whereas the time required to attain 180° was erratic enough to suggest that the rate of rotation beyond 135° is affected by other factors than temperature. Relative humidity was not one of these, since a test at 25° revealed no significant difference between 30% and 66% relative humidities.

The above findings suggested the advisability of considering the end-point of rotation 135° rather than the full 180°. Accordingly the five classes of rotation were arranged into three stages (fig. 1). *Stage I* are those which clearly have not begun rotation. *Stage II* are the intermediates between 0° and 135°. *Stage III* are the assumed sexually mature, with terminalia rotation equal to or greater than 135°.

In the final analysis only those temperature experiments were used in which 95% of the hourly samples eventually reached Stage III; the temperatures for the qualifying six experiments were 18°, 22°, 25°, 30°, 32°, and 36°. The results are given in table 1 at 3-hour intervals.

DISCUSSION

What factors determine the end-point of terminalia rotation can only be surmised. The observation that at completion one pleural line is continuous between the 7th and 8th segments while the other sometimes is not suggests a different amount of "give" in the sternum and tergum. There could also be differences among individuals in the elasticity of their intersegmental membranes. Induced copulations, using the McDaniel-Horsfall technique, demonstrated a persistence of flexibility or accommodation in the intersegmental membranes of mature males, so that copulation may bring a torsion of slightly over 135° around to 180°, where it may or may not remain after coitus.

Roth (1948) found males of *Aedes aegypti* (Linnaeus) attempting to copulate at 135° rotation, albeit unsuccessfully in most cases, while "many males with a slightly less than 180° rotation were able to copulate successfully." Copulating methods are very different in *Culiseta inornata* (Williston), studied by Rees and Onishi (1951). Males first copulated when 6 to 12 hours old, the terminalia rotation then being approximately 180°. However, complete rotation was not necessary; the minimum observed age at successful copulation was 3 hours and 50 minutes when the rotation was over 90° but not complete. Rotation usually started at 45 to 60 minutes of adult age, proceeded quickly to 90°, and slowed down thereafter. In our experiments with *Aedes taeniorhynchus* we also noted a deceleration in terminalia rotation beyond 90°. The *C. inornata* observations suggest that in mosquito species in which coitus is prolonged and not in flight, copulation is possible (though not necessarily occurring) earlier through quicker terminalia rotation. *Deinocerites cancer* Theobald is of this type, and one experiment at 25° showed its rate of terminalia rotation to be much faster than that of *A. taeniorhynchus*. The comparison may be summarized as follows:

	D. cancer	*A. taeniorhynchus*
50% start rotation	2 hrs	6 hrs
100% start rotation	5 hrs	8 hrs

The earliest observed copulation in caged *D. cancer* at room temperature was at 7 hours of male adult age (Haeger and Phinizee 1959).

Whether in mosquitoes the ability to copulate is attained in synchrony with the ability to ejaculate is a matter presently under study. We need only remark here that in *Aedes taeniorhynchus* the two requisites to successful mating appear fairly synchronized (Lum 1961). It is therefore justifiable to refer to males with Stage III terminalia as mature. This is especially so in dealing with populations when Stage III includes all from 135° to and including the full 180° rotation.

The data (table 1) suggest that percent unrotated in a sample should give a good age indication at temperatures up to 30°. The minimal age at beginning of rotation was 9 hours at 18°, 4 hours at 25°, and 2 hours at 32°. The percent with some degree of rotation (II + III) is clearly temperature related at 6 hours of age, being 0, 9, 58, 66, 79, and 82 at the six increasing temperatures between 18° and 36°. At 12 hours virtually all have begun rotation at temperatures above 22° and at 24 hours all but a few at 18° have begun rotation. The age at which 50% of the males had begun rotation was 16 hours at 18°, 11 hours at 22°, 6 hours at 25°, 5 hours at 30° and 32°, and 4 hours at 36°. Up to 24 hours of age, therefore, males can be aged by percent having begun terminalia rotation.

The attainment of maturity, as reflected in percent reaching Stage III, is also temperature related but again clearly so only up to 30°. Minimal ages at terminalial maturity at 18°, 22°, 25°, 30°, 32°, and 36° were 29, 15, 13, 6, 7, and 6 hours; and the ages at which 50% of the males reached maturity were 38, 23, 20, 11, 15, and 12 hours; the 95% level was not reached at 18° but for the remaining five temperatures it was reached at 42, 33, 30, 27, and 30 hours. Since (1) the majority of mosquitoes were over 12 hours of age before attaining Stage III, even at the highest temperatures, and since (2) mean air temperatures over 30° for longer periods than 12 hours are very rare in Florida at least, the experimental data for 32° and 36° represent unnatural situations. It should then be possible to determine the age of a sample by percent Stage III, and this would be applicable especially for ages between 12 and 36 hours.

The field picture is always more complicated than the laboratory experiment. In the experiments here discussed the emergence time was always known to be within a 5-minute period; in a natural brood of

Table 2.—Age determination in male *A. taeniorhynchus* by rotation of terminalia, using percentage in Stage I (nonrotated), Stage II (0°-135°), and Stage III (over 135°).

Mean temp. ° C.	Hours of adult age when: Percent Stage I										Percent Stage III									
	10	20	30	40	50	60	70	80	90	100	10	20	30	40	50	60	70	80	90	100
18	20	19	18	17	16	15	13	11	9	6	30	32	34	36	38	41	44			
20	17	16	15	14	13	12	11	10	8	5	23	25	27	29	31	33	37			
22	15	13	12	11	11	10	9	8	6	3	17	19	21	22	24	25	27	32	36	
24	10	9	8	8	7	7	6	5	4	3	14	17	19	20	21	22	24	27	32	
26	8	7	7	6	5	5	4	4	3	2	12	13	17	17	18	19	20	22	28	
28	8	7	7	6	5	4	4	3	3	2	10	12	14	14	15	15	16	18	24	
30	7	7	7	6	5	4	3	3	2	2	7	9	9	10	11	12	12	14	21	29

	Stage II				
	> 90%	> 80%	> 70%	> 60%	> 50%
18	23 - 30	19 - 31	18 - 32	17 - 37	16 - 40
20	19 - 24	16 - 25	15 - 26	14 - 32	13 - 32
22		14 - 19	13 - 21	12 - 22	11 - 24
24		10 - 17	9 - 19	8 - 20	7 - 22
26				6 - 17	6 - 18
28				6 - 13	5 - 14
30				6 - 9	5 - 10

A. taeniorhynchus, it is known only that 83% of a day's emergence will occur within a 9-hour period and 48% within a 3-hour period (Nielsen and Haeger 1955). In the experiments the temperatures were constant by room control; in the field, temperatures fluctuate and vary greatly within small distances in the area of massed resting at the emergence site. In studies of natural behavior, e.g., mating (Nielsen and Evans 1960) and dispersal (Nielsen 1958; Haeger 1960; Provost 1960) it is nevertheless important to know temperature and age of observed brood (= time since peak of emergence). If one of these is known the other can be calculated by measurements of male terminalial rotation. In order to facilitate the technique, the data from the experiments have been converted to table 2. Depending on temperature, it may prove necessary or convenient to use percent of the field sample in either Stage I or Stage III. The percent in Stage II, especially if high, can be very helpful. In most cases the table will permit a double check on age determination. It would take much more data than we had to establish the statistical deviations. The table will be useful, nevertheless, with samples as large as (20) or larger than ours. An example of the use of the table is given in table 3, based on data from Provost (1960). In this case, at temperature 25°, the percent in Stage I gave the best age estimate under 12 hours and the percent in Stage III the best beyond 12 hours.

The age at which male *A. taeniorhynchus* reach sexual maturity, as affected by temperature, is shown in figure 2. During the main breeding season for this

Table 3.—Example of use for table 2. Data from field collections in Borrichia Slough emergence of 1958 (Provost 1960). Emergence peak was at 20h October 15. Mean air temperature during and for 1 day after emergence was 25°.

	Oct. 16								Oct. 17	
	0135	0230	0330	0430	0530	1200	1545	1810	1035	1810
No. ♂ ♂ collected	151	174	42	106	41	116	113	232	606	43
% Stage I	33	27	29	22	17	3	0	0	5	0
% Stage II	64	71	69	69	68	75	35	20	88	25
% Stage III	3	2	2	9	15	22	65	80	7	75
Age, in hours, according to table 2										
Using I	7-8	7-8	7-8	8-9	8-9	>9	>10	>10	>9	>10
Using II	8-20		9-19	9-19	9-19	10-17				
Using III	<13	<13	<13	<13	<13	15-16	19-23	25	13	26
Estimated age	8	8	9	9	9	15	19	25	13	26
Actual age	6	7	8	9	10	16	20	22	15	22

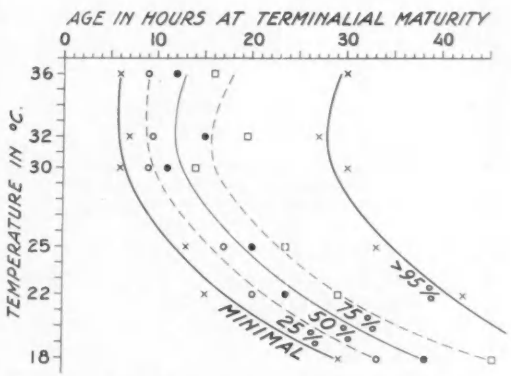

FIG. 2.—Age at terminalial maturity (= >135° rotation) as affected by temperature. Attainment of maturity at several sample percentages given to indicate variability.

mosquito in Florida, April to October, mean air temperatures over sufficiently long periods for maturity to be reached lie between 20° and 30°. At 20° the first male to reach maturity will be approximately 20 hours old, the majority will reach maturity at 29 hours of age, and nearly all at 48 hours. At 30° the corresponding three ages will be 6, 11, and 30 hours.

It is interesting that the temperature relationship to sexual maturation should so closely resemble that to pupal duration (Nielsen and Evans 1960, and fig. 3); at any temperature 3 hours of pupal duration corresponds to 1 hour of sexual maturation. Pre- and post-emergence maturation in *Aedes taeniorhynchus* are apparently affected by temperature in a parallel manner.

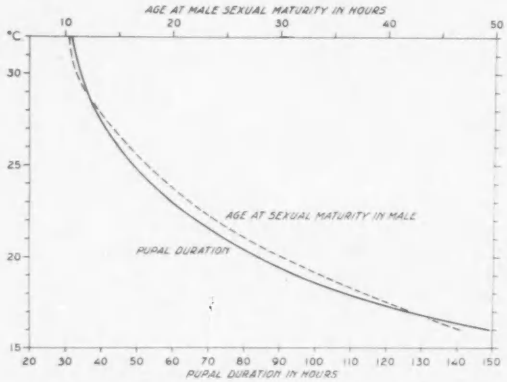

FIG. 3.—Comparison of pupal duration (cf. Nielsen and Evans, 1960) and age at sexual maturity in male as affected by temperature.

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A Study of the Odoriferous Glands of *Scaptocoris divergens* (Hemiptera: Cydnidae)[1]

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ABSTRACT

The stink glands of the adult and nymph of the fossorial cydnid *Scaptocoris divergens* are described. Preliminary mass spectrometric analysis indicates that the secretion is a mixture of a series of 7 aldehydes, 2 furans, and 2 quinones. The secretion is probably used principally as a defense against predators. In petri-dish tests the vapors of the secretion have a fungistatic or fungicidal action against *Fusarium oxysporum* f. *cubense*, confirming Timonin's original observations. Factors which may influence survival of *Scaptocoris* in certain types of soil are discussed.

Timonin (1957, 1958, 1961) found *Scaptocoris divergens* Froeschner[2] feeding on roots of banana and six other plants in Honduras. He showed that in petri-dish tests living *Scaptocoris* adults inhibited or killed the fungus *Fusarium oxysporum* f. *cubense* (E.F.S.) Sny. and Hans., the causative agent of *Fusarium* wilt of bananas, as well as 14 other soil-inhabiting fungi. Pot tests also indicated that the presence of *Scaptocoris* reduced the number of micro-organisms in the soil. Timonin concluded that an odorous substance emanating from the insects was responsible for the fungistatic or fungicidal activity.

Scaptocoris castanea can become a pest on sugar cane, of sufficient importance to require insecticidal control (Andrade and Puzzi 1951, 1953; Puzzi and Andrade 1957). Costa Lima (1940) also claimed

[1] Accepted for publication March 27, 1961.

[2] Mistakenly recorded as *Scaptocoris talpa* Champion.

that this species damaged tomatoes and pimentos in Argentina. However, Timonin (1961a) states that in pot tests, banana plants (var. Gros Michel) grown in soil containing *Scaptocoris divergens* or *Scaptocoris* and *Fusarium* were taller and developed better foliage than the plants grown in the *Fusarium* infested soil or the control plants.

Large areas of banana lands have been abandoned because of *Fusarium* wilt but in some abandoned areas there are "islands" of plants that are still in production. Timonin (1958) found *Scaptocoris* feeding on the roots of these productive plants. In one area in Guaruma II (La Lima, San Pedro Sula, Honduras), Timonin (1957a) found 16 *Scaptocoris* in 50.4 cubic feet of soil (0.3 insect per cubic foot) around the roots of a banana plant showing definite *Fusarium* wilt symptoms as compared with 532 insects in 7 cubic feet of soil (76 insects per cubic foot of soil) around the roots of an apparently healthy plant only 50 feet from the diseased mat. According to Timonin, soil surveys in Honduras showed that *Scaptocoris divergens* were generally found in greater numbers where there was relatively little *Fusarium* wilt (long-lived soils) whereas wilt-disease-abandoned areas or farms in which the disease was abundant (short-lived soils) contained few or no *Scaptocoris*. Timonin (1960), in a brief survey of farms in Guatemala, also claimed that this relationship held and that the soils in which few or no bugs were found (considerable abandoned areas) were of a heavier type than soils (loam to fine sandy-loam) in which the insects were more numerous and in the latter there was little or no *Fusarium* wilt. Unfortunately no quantitative studies have been made on the distribution of *Scaptocoris* in different soils but, in spite of this, there apparently is a correlation between the presence of *Scaptocoris* and certain soil types.

In March (dry season) Roy Roig (1960) conducted a survey of farms in Palmar, Costa Rica, for the presence of *Scaptocoris*. A total of 116 sections in 21 farms were surveyed. Of the general soil types examined there were 61 areas with sandy soil (i.e., very fine sandy loam to sandy clay loam) of which 54 (88%) contained *Scaptocoris*; the bugs were found at depths ranging from 4 to 57 inches. Of 48 clay soils (i.e., heavier in nature) examined none contained the hemipteron. Seven soils that consisted of layers of clay and sand contained no *Scaptocoris*. In the Esquinas seedbed in Palmar, Costa Rica, the writer found the bugs in sandy loam areas but not in heavier clay soils of the same farm; through the month of July of 1960 this region had 108.91 inches of rainfall yet the light sandy loam soils were not excessively wet when dug, whereas the heavier clay soils in the same farm were sticky. On farm 3 at Palmar, *Scaptocoris* was common in all sites dug in light-textured sandy-loam soils but was absent in the adjacent Mora farm where the soil is principally of a heavier clay type. The presence of *Scaptocoris* was correlated with light sandy-loam soil and in general these insects were found in soil that was considered relatively longlived in relation to *Fusarium* wilt.

Like many other Hemiptera, *S. divergens* emits an odor when disturbed or injured. Other species of this genus, namely *S. talpa*, and *S. giselleae* Carvalho also emit odorous secretions (Bianchi 1935; Carvalho 1952). In general, the defensive secretions produced by arthropods are stored in fairly large amounts in specialized glands. A knowledge of the glands could facilitate the identification of the odorous fluid which can be obtained with little contamination from other tissue by removing the reservoirs full of the secretion.

This paper presents the results of a study of *Scaptocoris* and its possible relationship to *Fusarium* wilt. Morphological studies were made of freshly dissected odoriferous glands in Ringer's solution and stained with methylene blue. Specimens were cleared in 10% KOH to show cuticular relationships. Glands were also fixed in Bouin's, sectioned, and stained with Heidenhain's iron haematoxylin, Delafield's haemotoxylin and eosin, or Mallory's triple stain.

Tests were made of the effect of the odorous secretion against *Fusarium* in petri-dish cultures, confirming Timonin's original findings. The defensive role of the secretion was determined using ants as predators. A preliminary analysis was made of the vapors of the secretion obtained from the gland reservoirs.

RESULTS

THE ODORIFEROUS GLANDS: *Adult Gland.*—In the adult (fig. 1A), the gland consists of a single large, wrinkled, membranous sac medially located at the base of the metasternum between the metathoracic coxae (fig. 1D). Associated with this storage reservoir are two large lobes or masses of cells, which lie free of the main body of the sac, but apparently open into it near its base. In addition to these lobes there are two rows of secretory cells appressed to the posterior surface of the reservoir (fig. 2B, arrows). The ventral nerve cord passes over the reservoir (fig. 1D) and presses down on the sac giving the reservoir the appearance of a bilobed structure. As a result of this pressure the posterior surface of a sac full of secretion lies against the sternites. When full of secretion, the turgid reservoir extends into the abdomen (fig. 1D); a full reservoir in a male measured 2.24 mm. wide and 1.65 mm. long.

The secretory lobes of *Scaptocoris* apparently correspond to the tubular glands in *Oncopeltus* described by Johansson (1957) but in the former there is no accessory reservoir associated with these secretory cells; the cells appressed to the reservoir in *Scaptocoris* correspond to the paired accessory glands which lie on the unpaired median reservoir in *Oncopeltus*.

Externally the secretion is emitted through two ostioles, one on each metapleuron (fig. 1B, arrows). These openings are clearly seen as small dark spots

at the end of a raised ridge along the metapleural plate (fig. 1C, arrow). This ridge is a hollow canal or vestibule through which the secretion passes after it leaves the reservoir (fig. 2A). At the base of the vestibule the membranous reservoir has a large muscle which upon contraction allows the secretion to pass up the vestibule and out of the ostioles. In the many instances in which we have seen the fluid released, the secretion flows out of the opening, spreads over the metapleural plate and evaporates. The metapleural plate differs from the surrounding sclerites. Anteriorly it is bordered by a groove and posteriorly by a flattened flange. There is a smooth V-shaped area above the ostiole and the remainder of the sclerite is comparatively roughly sculptured (fig. 1C). These modifications may serve to prevent the fluid from spreading to neighboring sclerites, as suggested by Johansson

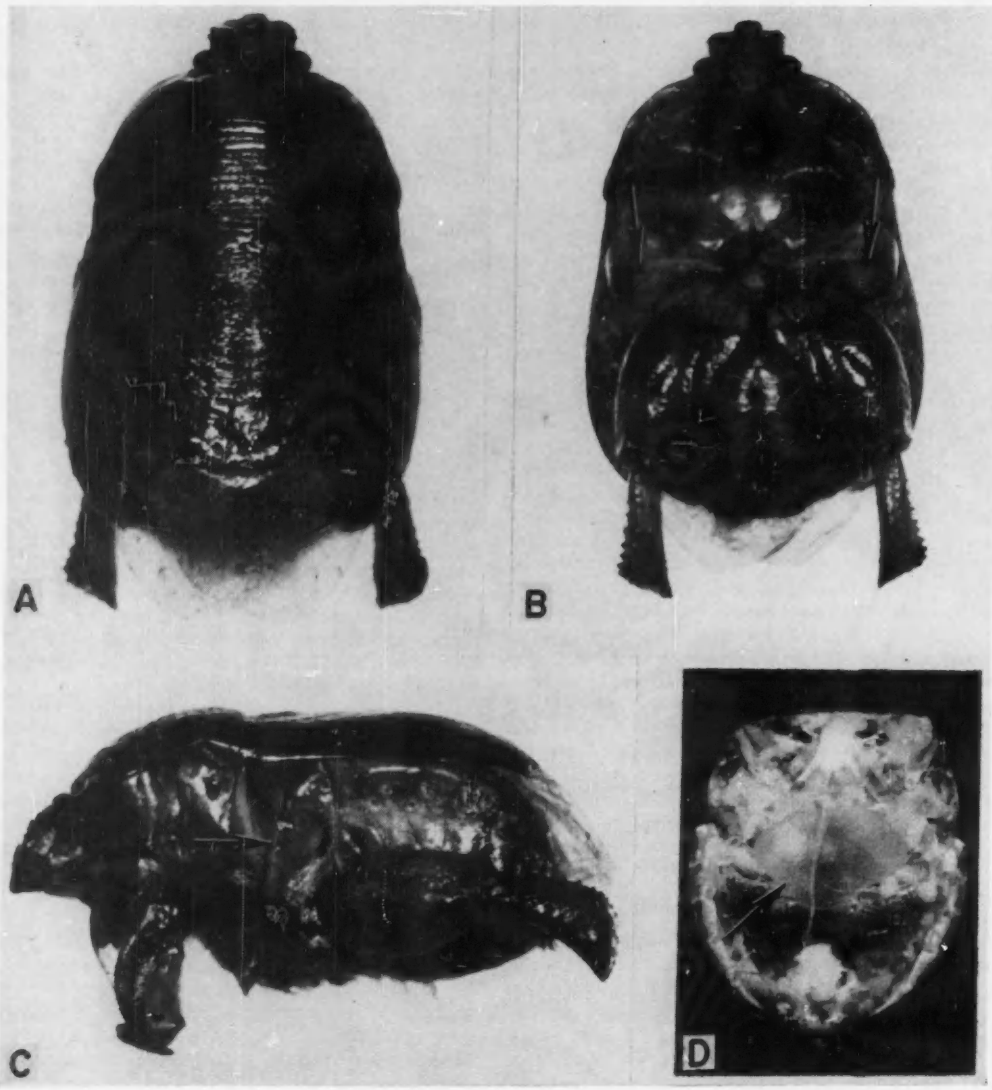


FIG. 1.—Adults of *Scaptocoris divergens*.
A. Dorsal view. B. Ventral view (mid-legs removed) showing the position of the gland openings (arrows) in the metapleural plate. C. Lateral view (mid-legs removed) showing the position of the gland opening (arrow). D. Dorsal dissection showing the reservoir (arrow) turgid with yellow secretion. Note the nerve cord leading from the thoracic ganglion over the surface of the reservoir.

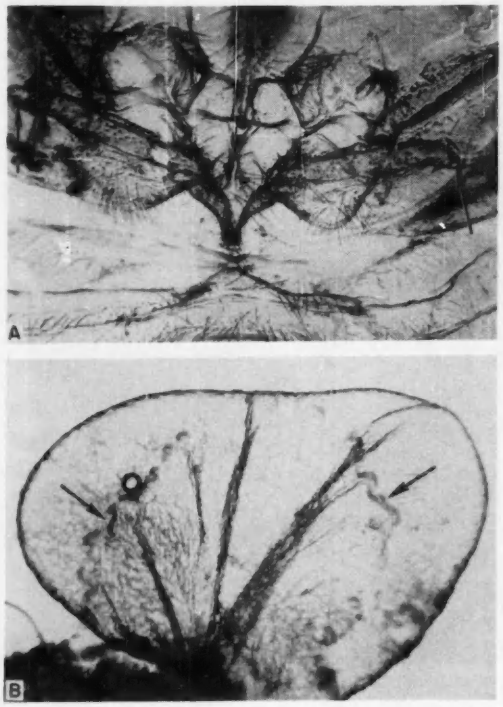


FIG. 2.—Parts of the odoriferous glands of adult *Scaptocoris divergens*.
A. Portion of the thoracic cuticle, cleared in KOH, showing the area in which the odoriferous gland was attached. Arrows point to the ostioles or openings through which the secretion is expelled. X 17.5. B. An entire reservoir or storage sac of an adult gland. In addition to the 2 lobes of secretory cells (not shown in photograph) which open into the base of the reservoir there are two rows of cells (arrows) which lie closely appressed to the surface of the reservoir. X 20.3.

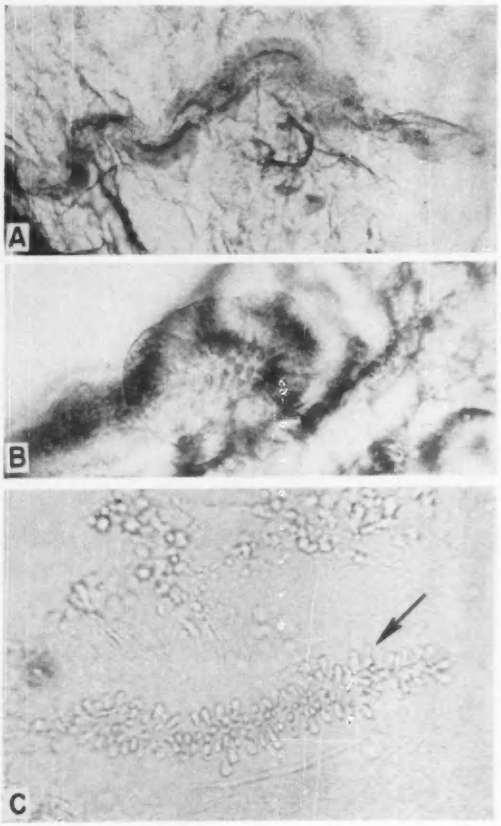


FIG. 3.—Part of the reservoir wall of an adult of *Scaptocoris divergens*.
A. Row of secretory cells closely appressed to the surface of the membrane. X 85.6 B. A secretory cell showing the canals in the central area. X 367.8 C. Part of wall of reservoir treated with KOH. The cellular tissue has been dissolved leaving the rows of secretory canals (arrow) visible. X 367.8.

(1957) for the functional significance of the sculpturing of the cuticle around the gland opening of *Oncopeltus*. Froeschner (1960) refers to this area as the evaporatorium.

The tubular glands consist of glandular cells distributed around long collecting tubes that open into the reservoir. Each gland cell possesses a secretory apparatus which consists of a clear vesicle in which a fine duct leads to one of the principal collecting tubes. Although the cellular tissue is colorless many of the long collecting tubes are filled with yellow secretion.

The secretory cells which lie appressed to the surface of the reservoir (figs. 2B, 3A) have a different and simpler collecting apparatus. In these cells there are apparently no vesicles and the secretion passes from the cell into the reservoir through small canals which are simply invaginations of the reservoir membrane (fig. 3B, C). By treating the reservoir with KOH the cells are dissolved leaving the canals clearly visible (fig. 3C).

Nymphal Glands.—In the nymph there are three gland reservoirs and their associated secretory cells, located in the abdomen. Each reservoir opens to the outside through a pair of dorsal openings between the third and fourth, fourth and fifth, and fifth and sixth tergites. The orifices to the reservoirs are on opposite sides of pale amber plates found on the median ventral margins of tergites 3, 4, and 5 (fig. 4A, arrows). The first gland reservoir, between the third and fourth tergites, is very small and usually has little or no secretion in it; in several tests in which nymphs were placed in concentrated sulphuric acid no secretion was seen to come from this reservoir. In one large nymph the second reservoir was 2.70 mm. wide and 0.54 mm. long; the third reservoir in this individual was 2.95 mm. wide and 0.78 mm. long. The reservoirs in these glands were relatively empty. When the sacs are not

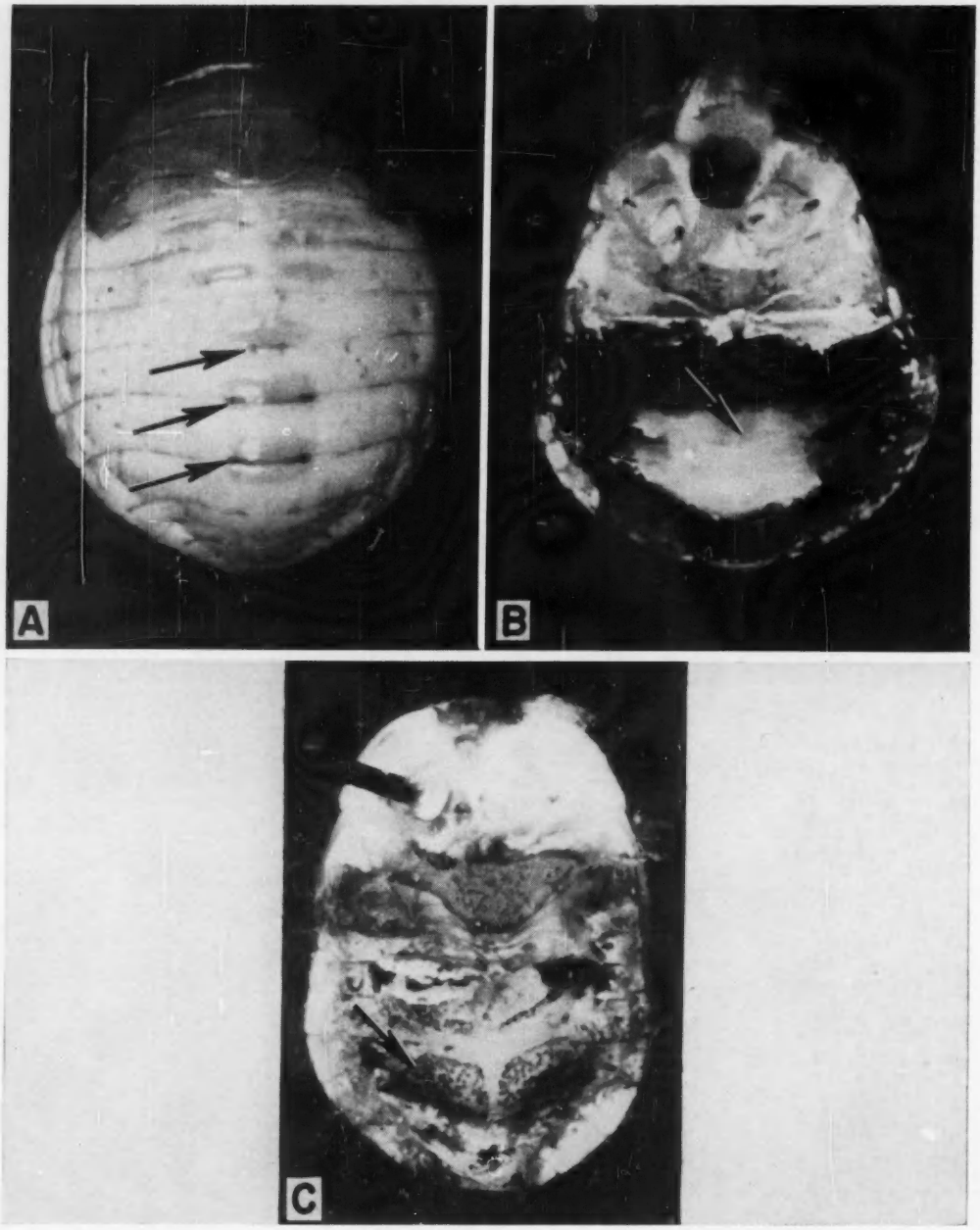


FIG. 4..—Nymphs of *Scaptocoris divergens.*

A. Dorsal view showing the median amber-colored areas indicating the positions of the tergal glands. Each arrow points to one of a pair of openings to the reservoir. B. Dorsal dissection showing 2 of the 3 abdominal reservoirs. Arrow points to the reservoir located between the fourth and fifth segments. The first gland (between the third and fourth segments) is very small and hidden by the second gland. C. Dorsal dissection, stained with methylene blue. The secretory apparatus stains blue (arrow) and the cells are seen to lie as a mat over the reservoir.

full of secretion they are boat-shaped and the largest reservoir (i.e., the third) extends almost the entire width of the abdomen (fig. 4B). When full, the second and third nymphal reservoirs are stretched and resemble the adult reservoir in shape. The sacs are attached medially to the tergites and fine muscles stretch them laterally and attach to the sides of the abdomen. There are muscles which attach near the reservoir openings and apparently regulate the ejection of the fluid. In addition there are other muscles which connect the reservoirs to one another (as in *Anasa tristis*; see Moody 1930).

The secretory cells lie appressed to the ventral surface of the reservoir; when the sac is full of secretion the secretory cells lie on the posteroventral surface (fig. 4C). In unstained preparations small, round, amber-colored, disklike structures are clearly visible (fig. 5A, B). These are the enlarged parts of the collecting ducts which lie in the vesicle (fig. 5C) in the secretory cell. The duct is a long coiled tube which leads into the reservoir; it is clearly demonstrated by dissolving the cellular tissue with 10% KOH, leaving only the canals and the reservoir. The secretion is a two-phased system of droplets floating in another liquid (fig. 4A).

Defensive Role of the Secretion.—Martorell (1939) found that when adults of *Scaptocoris divergens* were attracted to lights, they were readily eaten by the toad *Bufo marinus*. *Scaptocoris talpa* is said to be preyed upon by the larva of an elaterid beetle and in the laboratory the larva fed readily on this hemipteron (Bianchi 1935). Timonin (1957) also found a beetle larva that attacked all stages of *Scaptocoris divergens* in the laboratory. In spite of these observations, it is probable that *Scaptocoris* uses its odorous secretion as a defense against certain predators, especially those it might encounter in the soil. This was demonstrated by comparing the behavior of ants, *Pheidole* sp. (from La Lima, Honduras, and Palmar, Costa Rica), towards *Scaptocoris* which had or did not have the ostioles of the glands sealed; sealing was done with ferrule cement. The method of testing was similar to that used by Eisner (1958, 1958a) for demonstrating the protective role of the spray of *Diploptera* and *Brachynus*. The results are shown in figures 6 and 7 and it is evident that *Pheidole* is repelled by the secretion of nymphs and adults of this hemipteron. In an enclosed chamber such as was used in the experiments, the effectiveness of the secretion is enhanced since evaporation would be diminished and the odor could accumulate. For similar reasons the secretion would be a more effective repellent if ejected underground than when emitted above ground. Timonin (personal communication) has observed ants attack and carry off *Scaptocoris divergens* when the latter were placed on the ground; the writer has also seen this take place in plantations in La Lima. However, this does not negate the probability that under certain conditions (e.g., while burrowing through the soil or in confined cells in the soil) the secretion will act as a deterrent to predators which may encounter *Scaptocoris*.

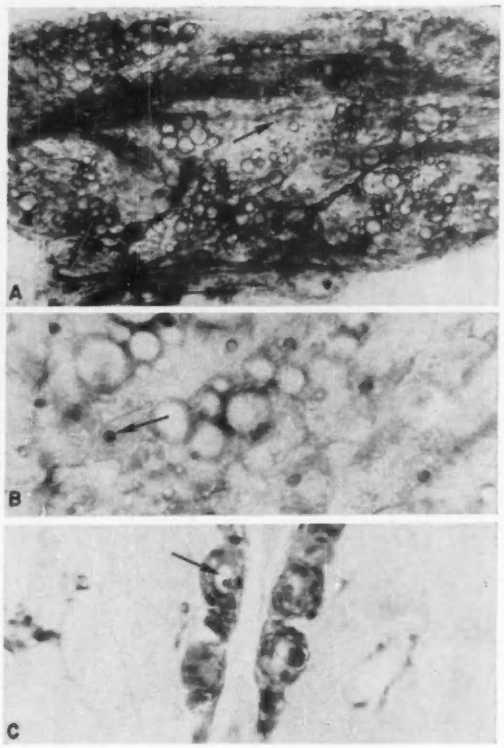
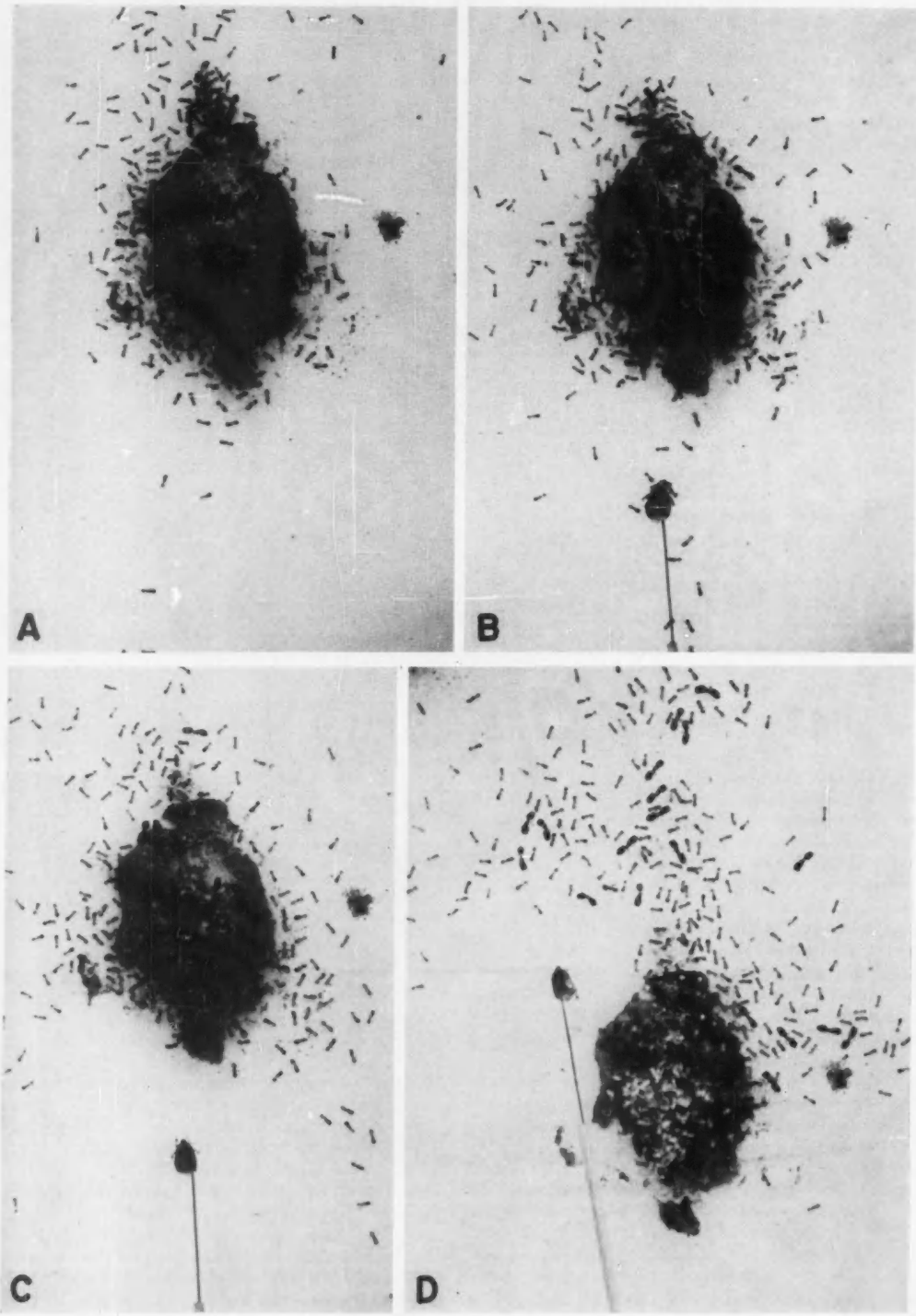


FIG. 5.—Glands of nymphs of *Scaptocoris divergens*. A. Entire gland, unstained. The round globules are droplets of secretion seen through the membranous wall. The small dots scattered over the surface (arrow, center) are the amber-colored swellings of the collecting tubules. Arrow, lower left, points to one of the openings from the reservoir. X 53.6. B. Surface view of gland cells, unstained, showing the amber-colored bulbs (arrow) of the collecting tubes. X 230.1. C. Histological section through a nymphal reservoir showing several secretory cells lying against the membranous sac. The arrow points to a clear, intracellular vesicle in which is found the amber-colored portion of the collecting tube. X 230.1.

Fungicidal and Fungistatic Action of the Secretion of Scaptocoris.—Timonin has shown that the presence of *Scaptocoris* in petri dishes inhibited or killed *Fusarium* depending on the number of insects present per dish. We have repeated with some modifications Timonin's laboratory tests to prove that it is the odorous secretion which is responsible for the fungicidal or fungistatic activity (table 1). When the openings to the glands were sealed to prevent escape of the secretion no inhibition of the fungus occurred; both adult males and females produced the inhibitory material. The secretion from nymphs also inhibited *Fusarium* although inhibition was not as complete as was obtained with adults. This was undoubtedly due to the fact that less secretion was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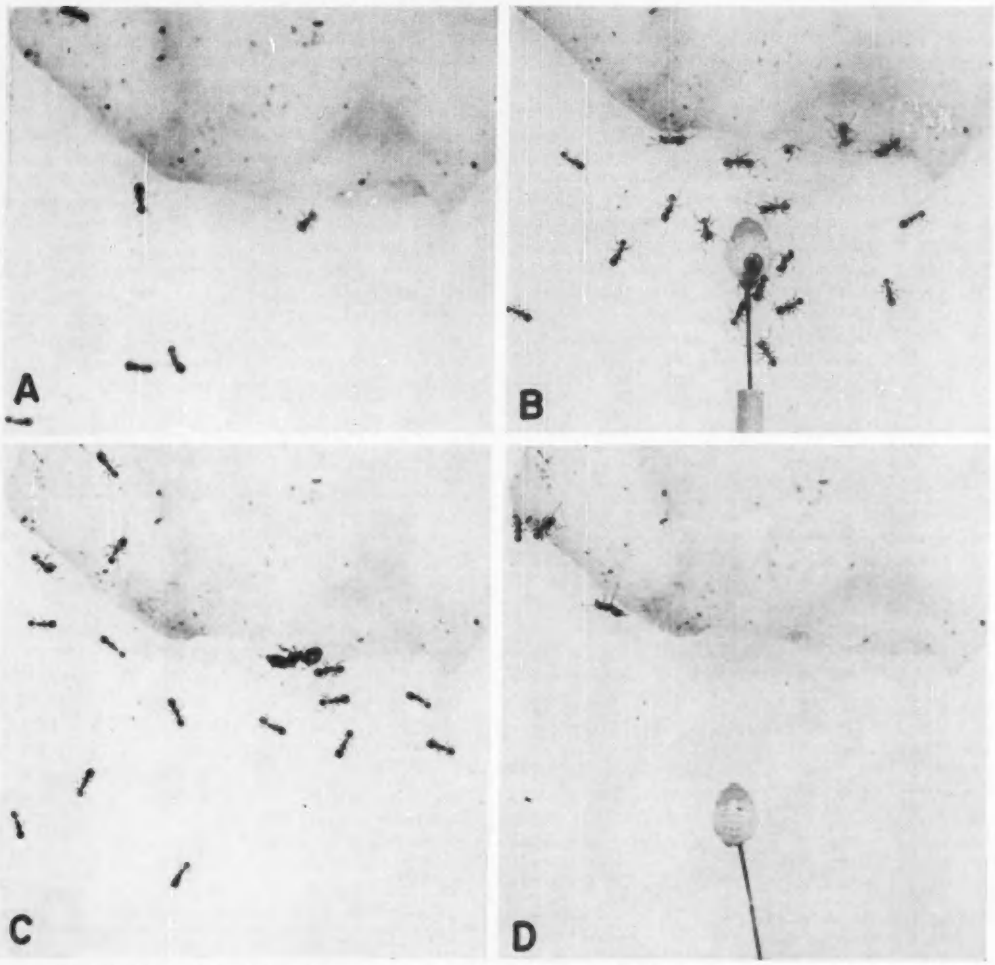

FIG. 7.—Repellent effect of the odorous secretion of a nymph of *Scaptocoris divergens* on individuals of a small colony of *Pheidole* sp. The closed chamber was similar to that used in the experiment in figure 6.
A. Distribution of ants prior to introducing the nymph: most of the ants were hidden under the moist cotton. B. A *Scaptocoris* nymph which had the openings to the dorsal glands sealed was introduced into the chamber. The hemipteron was under attack by the ants, many of which came out from under the cotton. C. Distribution of ants prior to introducing a nymph with its gland openings unsealed. D. The same as C shortly after introducing a nymph whose gland openings were unsealed. The ants were repelled and retreated under the cotton.

FIG. 6.—Repellent effect of the odorous secretion of an adult of *Scaptocoris divergens* on individuals of a colony of *Pheidole* sp. The ant colony, with brood, were on moist cotton in an enclosed chamber.
A. Undisturbed colony prior to introduction of *Scaptocoris*. B. An adult *Scaptocoris* with the external openings of its odoriferous glands sealed was mounted on a pin and introduced (lower center) into the chamber. Minor workers immediately left the brood and attacked the hemipteron. C. An adult *Scaptocoris* with the gland openings unsealed was introduced into the chamber. The ants were repelled. D. The same as C only the *Scaptocoris* was pushed farther into chamber. The ants have begun to pick up their brood and move them out into adjacent tubes.

elaborated by the nymphs; dissections of many nymphs have indicated that in most individuals the glands have relatively little secretion in them. Thoraces of the adults, containing the glands, inhibited *Fusarium* whereas the heads and abdomens of the same individuals did not. Additional proof that the secretion is capable of inhibiting *Fusarium* is shown in table 2 where material from isolated glands was used; the extent of inhibition varied with the relative amount of fluid present in the reservoirs. The six dishes in which no growth of mycelium was observed after 48 hours' incubation were kept for 6 days in which time only 2 of the 18 mycelial inocula (one in each of two petri dishes) grew; in one of these dishes the impregnated filter-paper disk had been removed after the 48-hour examination. The four dishes containing spore inocula were also kept for 6 days. In two of these the filter-paper disks were removed after 48 hours of incubation; after 6 days spores had germinated in one and not the other. In one of the remaining two dishes, the disk was removed after 72 hours and the spores germinated; in the last, the disk was left until the sixth day at which time there was sparse germination. The secretion from *Scaptocoris* may be fungicidal or fungistatic against spores or mycelia of *Fusarium*, the extent of the activity being determined by the amounts of secretion present.

Table 1.—Effect of the odorous secretion of *Scaptocoris divergens* on growth of *Fusarium oxysporum* f. *cubense* (Clone "C")[a].

Experimental conditions	Number of colonies: Inoculated	Number of colonies: Developed	Colony diameter in mm. (Mean ± standard error)
Control (fungus only)	20	20	19 ±0.2
Adult females (glands sealed)	12	12	17 ±0.6
Adult females (glands unsealed)	12	1	0.9±0.9
Adult males (glands unsealed)	12	0	0
Thoraces (containing glands) of five adults, males and females	4	1	1 ±0.8
Heads and abdomens of the above males and females	4	4	15 ±1
Nymphs (glands sealed)	12	12	17 ±0.4
Nymphs (glands unsealed)	12	6	6 ±2

[a] Four fungal colonies (mycelium) per dish; incubated 48 hours at 25°-28° C.; five *Scaptocoris* per dish or 0.8 insect per cubic inch of air space; fungi were grown on Czapek's agar.

The cockroach *Eurycotis floridana* ejects 2-hexenal when disturbed (Roth et al. 1956) from a large gland located ventrally in the abdomen (Stay 1957). Certain predators are repelled by the secretion (Eisner et al. 1959) and the compound also has bactericidal activity (Valcurone and Baggini 1957). This secretion was tested against *Fusarium oxysporum* f. *cubense*. Four dishes with mycelial inocula (four colonies per dish) and four dishes with a heavy concentration of spore inocula were used. Adult cockroaches (one per dish) were anesthetized and they ejected the secretion as they were immobilized. Several droplets of the secretion were absorbed on filter-paper disks and one disk was placed inside the cover of each of the inverted petri dishes so that only the vapors were in contact with the fungi. Readings were made after 48 hours (cultures kept at 28° C.). There was no mycelial growth in any of the dishes exposed to the 2-hexenal obtained from *Eurycotis*. Three of the four dishes containing the spore inocula showed no germination; the spores germinated in one dish. In the control dishes all of the mycelial inocula grew; mean diameter of the 16 colonies (four dishes) was 17.0 ± 0.3 mm. All of the four control dishes with spore inocula showed dense growth.

Table 2.—Effect of the odorous secretion of *Scaptocoris divergens* on growth of *Fusarium oxysporum* f. *cubense* (Clone "C")[a].

Experimental conditions	Number of colonies: Inoculated	Number of colonies: Developed	Colony diameter in mm. (Mean ± standard error)
Control (fungus only)	9	9	18±0.3
Gland reservoirs full of secretion:			
Males	6	0	0
Females	12	0	0
Gland reservoirs partly filled with secretion:			
Males	3	3	5±0.8
Gland reservoirs with little or no secretion:			
Males	6	6	14±1
Females	3	3	15±0.5

[a] A single adult gland reservoir containing the secretion was dissected out, crushed on an antibiotic filter paper disk, and placed in the petri dish below the agar containing the *Fusarium* inocula (mycelium). Three fungal colonies per dish; incubated 48 hours at 25°-28° C.

In addition to the mycelial inoculations, four petri dishes were inoculated with heavy spore suspensions. A disk of filter paper on which a single male gland full of secretion was crushed was placed in each dish. No germination occurred 48 hours after inoculation; two control dishes had dense mycelial growth after the same period of time.

Preliminary Analysis of the Secretion of Scaptocoris.—A preliminary examination of the defensive secretion was made by M. Bazinet, of the Pioneering Research Laboratories, U. S. Army Quartermaster Laboratories, Natick, Mass., using a Consolidated 103 C Analytical Mass Spectrometer. The first sample analyzed consisted of an aliquot of the vapors from 15 gland reservoirs dissected from adult females. This analysis of material fractionated at −145° to −40° C. indicated the presence of a series of seven aldehydes, namely, propenal, propanal, butenal, 2-hexenal, pentenal, heptenal, and octenal. In addition the spectrum showed the presence of three or four other compounds in the secretion. A second analysis made of the vapors from a *single*

adult gland showed the same series of aldehydes and in addition indicated the presence of four other compounds, namely furan, methylfuran, methylquinone, and another unidentified quinone.

The identification of the first four members of the aldehyde series indicated was accomplished by comparing the spectra obtained from the distilled fractions to standard reference spectra on file at the Quartermaster Laboratories. Associated isotope ratios in the parent mass region of the fragmentation patterns obtained from these fractions suggest the presence of the last three members, pentenal, heptenal, octenal, and the furans and quinones. The lack of reference spectra and the presence of these latter compounds in trace quantities prevented their positive identification.

Further insight into the characterization of this mixture was realized by sampling another aliquot of the vapors from the 15 glands on the mass spectrometer. A low ionizing voltage spectrum (Bazinet and Merritt 1959) was obtained which helped to simplify the complex mixture by showing only the parent masses for the listed compounds.

Miss Alice McCarthy, formerly of the Quartermaster Laboratories, subjected a 5-cc. volume of air obtained from the original sample from 15 adult glands used by Mr. Bazinet for mass analysis to gas chromatographic analysis and obtained 5 peaks. One of the compounds had a retention time value of 10.9 which was similar to that of synthetic 2-hexenal (retention value 10.8).

The secretion in the gland reservoirs of *Scaptocoris* is yellow in color. When the glands are carefully dissected (so that the reservoir is not punctured) in saline solution and left overnight, the yellow compound(s) diffuses through the membranous wall leaving the colorless water-insoluble compounds in the sac; the water-insoluble compounds are soluble in carbon tetrachloride, ether, and chloroform.

DISCUSSION

The odorous secretion of *Scaptocoris divergens* is probably principally a defensive mechanism against predators. It is fungicidal or fungistatic against spores and mycelium of *Fusarium oxysporum* f. *cubense* in petri-dish tests, as first shown by Timonin. A number of arthropod secretions (quinones, aldehydes) have been found to be fungicidal or bactericidal (Estable et al. 1955; Pavan 1953, 1959; Valcurone and Baggini 1957), and it is not surprising that the *Scaptocoris* secretion which apparently consists principally of a series of aldehydes should inhibit or kill *Fusarium* in petri-dish tests.

The yellow color of the secretion may be due to the presence of one or more *para*-benzoquinones; all the known cases of quinones used in defense by arthropods have been *para*-benzoquinones (Pavan 1959). *Para*-benzoquinone derivatives have been found in beetles (Alexander and Barton 1943; Loconti and Roth 1953; Schildknecht 1957; Blum and Crain 1961), a cockroach (Roth and Stay 1958), an arachnid (Fieser and Arado 1956), and millipedes (Béhal and Phisalix 1900; Barbier and Lederer 1957; Trave et al. 1959). Of the unsaturated aldehydes indicated as being present in *Scaptocoris*, 2-hexenal has previously been recorded from three species of cockroaches (Roth et al. 1956; Roth and Willis 1960; Chadha et al. 1961) and four species of pentatomids (Waterhouse et al. 1961; Blum 1961). The compound 2-octenal is also present in the defensive secretions of species of pentatomids (Waterhouse et al. 1961). Trans-2-heptenal is found in the odorous secretion of a pentatomid (Blum et al. 1960).

The secretion of *Scaptocoris* may be fungicidal or fungistatic to *Fusarium* in petri-dish tests, but it is still unknown whether *Scaptocoris* affects the *Fusarium* population in the field. Apparently the bug gives off the secretion only when it is disturbed (i.e., when the soil is disturbed or when the insect is attacked by a predator). It is difficult to imagine a sufficiently large population of *Scaptocoris* giving off enough secretion, frequently enough to reduce the *Fusarium* population under natural conditions. Timonin (1957, 1961) found, in pot studies, that the presence of *Scaptocoris* reduced the number of *Fusarium* in the soil. It should be remembered, however, that "After the addition of fungus inoculum and/or insects, the soil was thoroughly mixed by rotating and shaking the jars so that a homogeneous distribution of inoculum and insects was obtained." (Timonin 1961). This treatment stimulates the insects to release the secretion which would reduce the initial fungal population. But this type of stimulation and homogeneous distribution does not occur in nature. It would be of interest to determine the number of fungi present in these pots over a period of time, once the bugs were well established and had remained undisturbed; Timonin made spore counts only during the first 6 days of incubation.

Although all evidence indicates that these secretions are given off only after the insect is subjected to some external traumatic stimulus, one cannot dogmatically exclude the possibility that some material may leak out from the glands when the insect is not attacked. It should also be remembered that in the case of nymphs, the cuticular linings of the gland reservoirs, together with the defensive secretions, are shed at each molt and it is conceivable that when this occurs the liberation of the fluid from the shed cuticle may affect the microflora in the neighborhood of the shed skins. How important this is in affecting the microbial population of the soil in nature is unknown.

To determine whether *Scaptocoris* could harbor *Fusarium oxysporum* f. *cubense*, Timonin (1957, 1961) washed six of the Hemiptera in sterile water, macerated them under aseptic conditions and plated them on agar; no *Fusarium* were cultured. This was repeated with insects that had been placed in soil heavily inoculated with *Fusarium*. Again results were negative. Timonin concluded that *Scaptocoris*

"is not a carrier of *F. oxysporum* f. *cubense* externally or internally." Timonin apparently neglected the fact that in macerating the insects the secretion from the bugs is liberated and would kill any *Fusarium* if it were present. To prove that the fungus is not present inside the insect, the contents from various parts of the digestive system would have to be cultured after being removed by dissection, care being taken that the reservoir of the defensive gland is not ruptured. The fact that water used to wash the bugs also failed to give positive results could have been due to the fact that the insects are induced to give off the secretion which could inhibit the fungus. The yellow component of the bug secretion is water soluble.

The recent study by Willis and Roth (1962) indicates that a number of factors may influence the survival of *S. divergens* in certain types of soil. The following discussion, which attempts to explain the apparent correlation between *Scaptocoris* and so-called long-lived soils, refers to data and observations in their paper.

The scythelike modification of the front legs of *Scaptocoris* (adults and nymphs) makes it possible for this insect to dig through soils of different texture and structure and their burrowing ability is independent of the cavity system of the soil. In laboratory tests these insects can dig into and through heavy clay in which they are not usually found in nature, provided there is sufficient moisture to allow the front tibiae to loosen the soil. The bugs are unable to dig into dry sandy loam (1% moisture) or in a very wet sandy loam (42% moisture) in which there is an excess film of water on the surface. Aside from these limitations, hardness of the soil is the only other principal deterrent to digging; clays hardened by drying cannot be penetrated. Adults and nymphs which burrow into heavy clay can become trapped in their cells if the clay hardens, making it impossible for them to dig. The rapidity with which the insects burrow varies with the type of soil, being slower in the heavier clay soil and faster in the sandy-loam soil.

In the field *Scaptocoris* adults and nymphs may be found in the soil some distance from roots. Nymphs apparently do not remain attached to a single root site during their developmental period. In starvation tests (insects kept in moist sandy loam) 50% of 20 adult males lived for 16 days, 45% of 20 adult females lived for 11 days and 50% of 10 nymphs lived 37 days. The ability to withstand starvation makes possible the migration of *Scaptocoris* through soil provided it is of the right consistency to permit the insects to dig.

An excess of soil moisture for prolonged periods of time is harmful for *Scaptocoris*. The majority of adults and nymphs die within 3 days when floating on water. Similar results were obtained by submerging adults in water or by flooding sandy-loam soils in which the bugs were present. Adults of *Scaptocoris* also are highly susceptible to desiccation when exposed to low relative humidities, and even in a high humidity the insects lose water. *Scaptocoris* requires a high humidity for survival and probably in the small cells in which the bugs are found in the soil, the air is saturated. At certain times of the year adults have been found attracted to lights on rainy nights. Flying only during rainy nights may be obligatory in that saturated air is necessary to prevent desiccation of the adults. Undoubtedly dispersal of the species over a large area occurs during this flight period.

Once in the soil, movement underground may be influenced by soil moisture content. Adults, in laboratory tests, are capable of discriminating between soils of different moisture contents and show a "preference" for the wetter soil.

Johnsgard (1957) in his study of soil types and *Fusarium* wilt in general correlated low incidence of the disease with soils of good natural drainage. High disease incidence is associated with areas which favor a considerable influx of ground water and with soils of poor permeability characteristics or poor effective drainage. Since the presence of *Scaptocoris* appears to be correlated with long-lived soils, it can also be correlated with well-drained soils. It is evident from the water relations of these insects that soil moisture could well be the limiting factor in the establishment of *Scaptocoris* in a particular area. The insects would survive for only a short time in soils that become waterlogged. If adults burrowed into heavy clay soils that hardened during the dry season they could become trapped in their earthen cells as a result of being unable to dig through the dried cementlike material. In well-drained sandy-loam soil, the insects would have the greatest opportunity of surviving for this soil would not become waterlogged and even if there was a tendency for the soil to dry out during the dry season, the insect is capable of readily digging downward toward soil of higher moisture content.

Kühnelt (1955) has stated that for assessing the water situation of the soil, the presence of drought-resisting species is not so decisive as the absence of species which require moisture. *Scaptocoris* is not a drought-resistant form and possibly the presence of this species may be indicative of well-drained, light-textured soils. After finding *Scaptocoris* feeding on roots of a banana plant growing on an "island" in an area abandoned because of *Fusarium* wilt, Timonin (1961) suggested that *Scaptocoris* might be a carrier of *Fusarium oxysporum* f. *cubense* or it might safeguard the plants from the disease. He concluded that *Scaptocoris* is an antagonist to *Fusarium*. However, there is a third possibility that must be considered, namely that *Scaptocoris* might be found principally in certain types of soils with characteristics that favor survival of the hemipteron but not *Fusarium*. Conversely soils which may be favorable for *Fusarium* may not support *Scaptocoris*. Perhaps the presence of *Scaptocoris* may be an indicator of certain long-lived soils. But it does not

follow that *Scaptocoris* is responsible for the long life of the soil.

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Weight and Hatchability of *Aedes aegypti* Eggs (Diptera: Culicidae)[1]

JOHN W. KLIEWER[2,3]

ABSTRACT

At laying, eggs of *Aedes aegypti* (L.) weighed about 5 μg. As the embryo developed and the eggs became conditioned, weight increased to 12 ± .03 μg. Conditioned eggs subjected to various relative humidities showed a progressive loss in weight and a corresponding decrease in hatchability. "Optimally" conditioned eggs held at 0% relative humidity at 25° ± 2° C. decreased in weight to near 3.5 μg in 72 days at which time hatching ceased. Suboptimally conditioned eggs of the same initial weight decreased to the same extent in about 55, 80, 150, 205, and 230 days in relative humidities of approximately 0%, 30%, 60%, 75%, and 90% respectively. A high correlation between the changes in weight and hatchability is demonstrated.

On the basis of egg physiology, mosquitoes may be divided into two groups, viz., those producing eggs which are at least facultatively diapausing and those producing nondiapausing eggs. Nondiapausing eggs develop and hatch immediately. They must, therefore, be in close proximity to water to enable the larvae to survive. *Culex* mosquitoes, which lay eggs in rafts, and *Anopheles* mosquitoes, which lay eggs singly on the water surface, are examples of those which produce nondiapausing eggs.[4] Among those which produce diapausing eggs are the aedine mosquitoes. *Aedes* mosquitoes do not lay their eggs on the water surface; they are deposited in moist areas and are normally in contact with such moisture for several days. During this time they develop a resistance to desiccation and can thereafter survive long periods of time in the absence of moisture.

There are differences of opinion as to how *Aedes* eggs become resistant to a dry atmosphere and the length of time the eggs remain viable under conditions of different humidities. Further, there is an almost total lack of information on the change in weight of *Aedes* eggs from the time of laying through development and conditioning to the time when they no longer hatch.

The purposes of this study were to determine (1) the change in weight of *Aedes aegypti* (L.) eggs from laying to the time when they become hatchable, (2) the changes in weight and hatchability of conditioned eggs of *A. aegypti* with time at several relative humidities, and (3) the extent of correlation of weight change (presumably water loss) and hatchability under laboratory conditions.

MATERIALS AND METHODS

Experimental Design.—A fairly evenly distributed series of relative humidities was selected, viz., approximately 0%, 30%, 60%, 75%, and 90% at a temperature of 25° C. Samples were weighed and tested for hatching at 4 days and at 10-day intervals thereafter as long as hatching occurred at that particular humidity. There were three replicates at each relative humidity. Egg sample sizes were between 100 and 150 eggs each.

Eggs.—A timed series of eggs, the ages of which were known to within 10 minutes, was collected and held on a moist substrate for weighing and observing development at intervals up to the point where they became hatchable. Observations on development were made by dechorionating the eggs with sodium hypochlorite after the method of Mortenson (1950). For desiccation experiments large numbers of eggs were collected from seven cages of randomized *A. aegypti* adults over a 3-day period. Strips of filter paper placed around the inside of finger bowls containing about 1 cm. of water were used as the egg-laying substrate. Eggs were randomized in distilled water 24 hours after the collection period, placed on filter-paper strips in small circular patterns of from 100 to 150 eggs each, and surface sterilized by impregnating the strips with a 1:1000 dilution of Roccal. Each circle of eggs, then, represented a sample of the entire colony population. The egg-sample strips were then stored on a moist substrate in the insectary to allow development and conditioning of the eggs. Temperatures throughout all phases of experimentation were approximately 25° C.

Relative Humidity Chambers.—Plastic zipper boxes (about 1 inch deep and 1 inch in diameter) containing saturated salt solutions were prepared so that each had a small chamber for the egg sample. Nonabsorbent cotton was used in each chamber to prevent the egg sample, which was on a small pad of filter paper about 1 cm. in diameter, from coming into contact with the solution or with the walls of the zipper box. The lid of each box had a small hole which allowed the air inside the box to come into equilibrium with the desiccator environment into which it was placed. Desiccators with an inside diameter of about 15 cm. were prepared with the appropriate saturated salt solution to receive the zipper boxes, three for each of the five different relative humidities. This arrangement provided an environment within an environment in which a minimum of disturbance to the eggs resulted when the desiccator lid was removed periodically. After

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[4] *Anopheles walkeri* Theobald, which lays diapausing eggs (Hurlbut 1938), is an exception; there may be others.

such disturbances equilibrium, it is believed, was soon reestablished. The desiccators were hermetically sealed.

SATURATED SOLUTIONS.—Saturated solutions of sodium tartrate, sodium chloride, ammonium nitrate, and calcium chloride and dry phosphorous pentoxide were used to maintain the relative humidities. These solutions and the dry P_2O_5 provide relative humidities of 92%, 75.5%, 62.5%, 29.5% and 0% respectively at 25° C., according to Winston and Bates (1960).

CONSTANT TEMPERATURE CHAMBER.—Saturated salt solutions maintain desired humidities only when the temperature is held relatively constant. The constant temperature box used was a well-insulated box kept in the laboratory where the temperature was maintained at 25° C. The temperature throughout the period of experimentation was 25° ± 2° C. This amount of fluctuation in temperature had no effect upon relative humidity in most cases and in no case was the resulting fluctuation in relative humidity greater than ± 2% of the stated value.

WEIGHING APPARATUS.—All weights were made using a Sartorius MPR 5 microbalance. Average weights were calculated by weighing the entire sample or by weighing the sample in lots of about 10 eggs each to obtain an estimate of the variance of the sample.

HATCHING STIMULUS.—Ascorbic acid was used as the hatching stimulus because it was shown in earlier experiments to be (1) an adequate and consistent stimulus, (2) sufficiently stable to remain unchanged throughout the period of the tests, and (3) one that would not cloud the water, thereby making recovery of the eggs difficult.

EXPERIMENTAL PROCEDURE.—Weight change from laying through conditioning was measured by removing the eggs from the moist substrate and weighing them in lots of 10 or more. Eggs, during this early stage, cannot resist desiccation. They were therefore transferred directly to the weighing pan and weighed. It was recognized that the evaporation of moisture from the eggs constitutes a source of error. By careful timing, however, reasonably accurate measurements were obtained. Consecutive measurements of such average weights were made at about 10-minute intervals during the first 2 hours, 1-hour intervals through the next 18 hours, and at 8-hour intervals through the final 60 hours, at which time the eggs were hatchable.

When eggs which had been held at different relative humidities were to be tested for weight change and hatchability, they were removed from their filter-paper substrate by means of a fine needle bent in the form of a loop. They were gently loosened from the paper and allowed to fall into the weighing pan. After weighing, the eggs were transferred to small stender dishes (inside diameter 5 cm.) and counted. About 35 ml. of distilled water and 25 mg. of ascorbic acid was added to each dish. Hatching counts were made 48 hours later at which time larvae were removed from the dish with a small pipette and discarded. Empty shells and unhatched eggs were also counted to ascertain the number hatched.

Embryos in aging eggs, particularly those subjected to low relative humidities, become weakened to the point where they do not emerge normally. There were all degrees of emergence from the egg shell from merely cracking the "lid" to complete emergence of the larva. If the larva was able to emerge halfway and if it appeared healthy it was counted as hatched. If, however, the larva had only cracked the shell or if it appeared abnormal it was not counted.

Two series of desiccation tests were made, one series with "optimally" conditioned eggs (i.e., those allowed to develop and condition on a moist substrate in an atmosphere of high relative humidity) and the other with suboptimally conditioned eggs which had been subjected to a dry atmosphere for a period of about 8 hours during development. The latter eggs were subjected to all five relative humidities; the "optimally" conditioned eggs were subjected to 0% relative humidity only.

An effort was made to determine whether eggs which did not hatch on the first attempt could be induced to hatch after additional conditioning treatment. Unhatched eggs of the "optimal" series were removed from the hatching medium, washed in distilled water and placed on moist filter paper to be exposed to the air in an atmosphere of high humidity for 48 hours. After this treatment the eggs were again subjected to hatching tests. At 48 hours hatching counts were made and unhatched eggs were discarded.

RESULTS

At the time of laying, eggs weighed approximately 5 μg. As can be seen in figure 1,[5] gain in weight is most rapid during the first 1½ hours, at which time the slope progressively decreased until the asymptote was approximated at about 16 hours. There was no significant change in weight from that time through the time when the eggs became hatchable. The mean weight from 16 hours through hatchability was $12 \pm .03$ μg.

Gross observations on the development of the transparent cuticle[6] and other embryonic structures were made by dechorionating eggs of known ages. Prior to the formation of the transparent cuticle the eggs readily collapsed in atmospheres of low humidity. That this envelope plays a major part in the waterproofing of the egg cannot be doubted, since it is at this time that the egg becomes most resistant to desiccation and since the gain in weight virtually ceases at the time it is completed.

Segmentation appeared to be complete in some eggs at 40 hours and in all at 53 hours. The egg-

[5] Points plotted on the graphs are means of the replicates.

[6] Beckel calls this embryonic envelope the transparent cuticle, Christophers refers to it as the serosa, and Telford calls it the vitelline membrane.

breaker was first apparent at 54 hours and by 83 hours all viable eggs possessed this structure. With the formation of the eggbreaker, embryonic development appeared complete. The first hatching was observed at about 60 hours; by 96 hours the percentage hatch was as high as could be expected at any subsequent time. When hatch was plotted against time, the resultant curve approximated a normal distribution.

Figure 2 shows the change in weight and hatch-

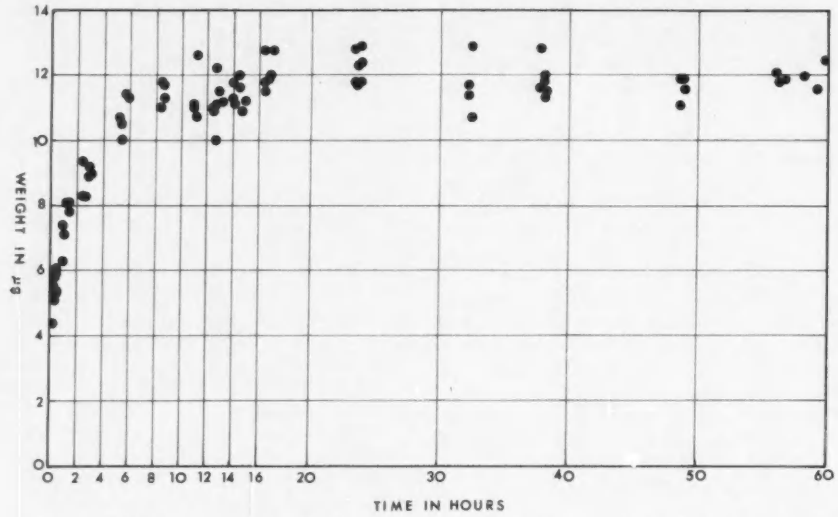

FIG. 1.—Change in weight of *A. aegypti* eggs from when they are laid until they become hatchable.

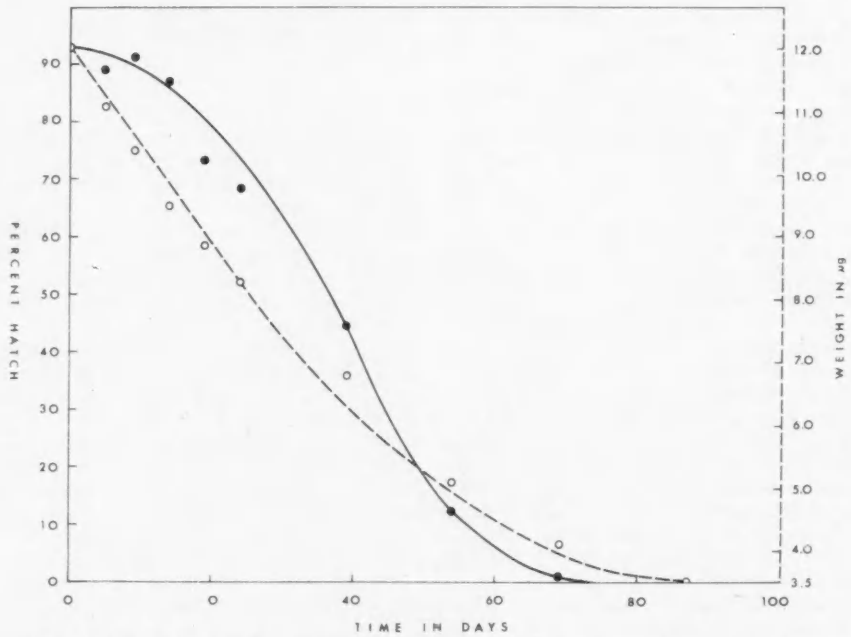

FIG. 2.—Comparison of changes in weight and hatchability of "optimally" conditioned eggs of *A. aegypti* when held at 0% relative humidity at 25° C.

ability of "optimally" conditioned eggs held at 0% relative humidity in the present study. Initially, the eggs weighed about 12 μg and decreased in weight to about 4.0 μg in some 72 days at which time hatching ceased; the initial mean hatch was 93%.

Change in weight of suboptimally conditioned eggs held at each of the five different relative humidities is shown in figure 3. Eggs decreased from the initial weight to near 3.5 μg at different intervals, viz., 55, 80, 150, 205, and 230 days, depending upon

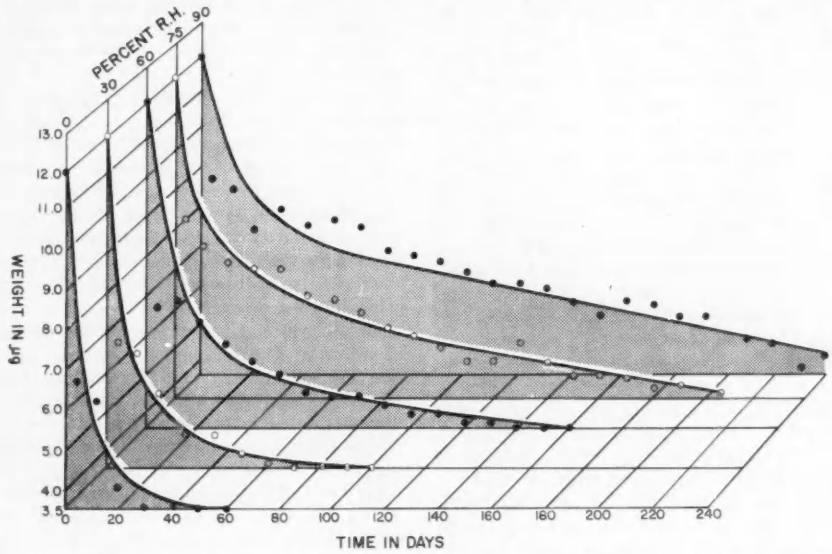

FIG. 3.—Comparison of changes in weight of suboptimally conditioned eggs of *A. aegypti* when held at different relative humidities at 25° C.

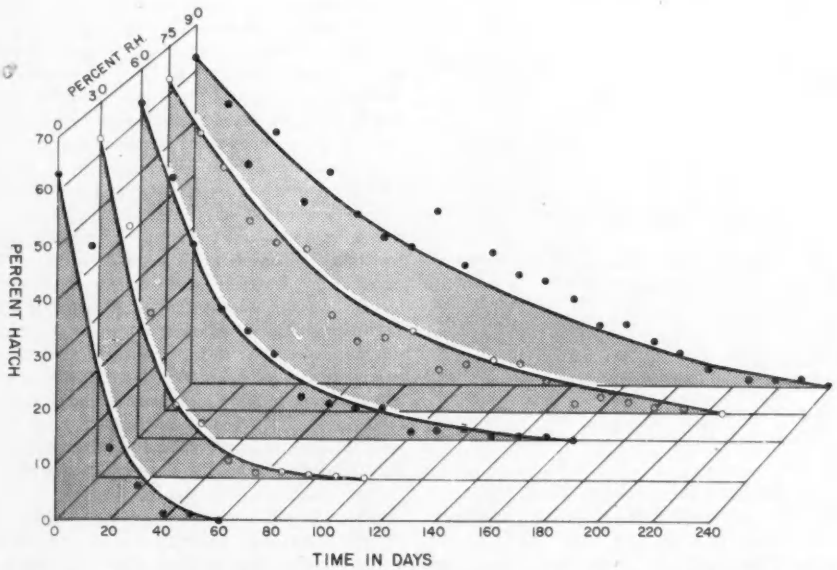

FIG. 4.—Comparison of changes in hatching response of suboptimally conditioned eggs of *A. aegypti* when held at different relative humidities at 25° C.

the relative humidity to which the eggs were subjected. There was no hatching of eggs which weighed this little.

Figure 4 shows the change in hatching response of the same suboptimally conditioned eggs held under the indicated conditions. The initial mean hatch was 63% and decreased to 0% after the intervals listed above.

It will be noted that the decline in weight is considerably more gradual in "optimally" (figure 2) than in suboptimally conditioned eggs (figure 3). In the latter case, the initial drop in weight is relatively abrupt. The most probable explanation for this abrupt weight loss is that those eggs whose embryos had only partially developed transparent cuticles were either killed or seriously damaged by the temporary deprivation of moisture during development. The eggs, however, absorbed water when again placed on a moist substrate and, when subsequently subjected to low humidities, quickly lost water and were reduced to collapsed shells. This rapid loss is reflected in the mean weights obtained on the first weighing 4 days after the start of the experiment. Eggs in which embryogeny has progressed to the point where the transparent cuticle was formed were not so greatly influenced by this temporary deprivation of water. They were adversely affected, however, since in no case did an egg hatch after about 55 days, whereas "optimally" conditioned eggs, hatched up to about 75 days at the same temperature and humidity.

Table 1 summarizes the coefficients of regression and correlation. In all cases the "r" value was highly significant which shows the importance of water relations of the environment to the viability of mosquito eggs.

Table 1.—Summary of coefficients of linear regression and correlation of "optimally" conditioned eggs held at 0% R.H. and of suboptimally conditioned eggs held at several relative humidities.

Relative humidity	Regression Coefficients: Weight on time	Regression Coefficients: Hatch on time	Correlation coefficients (Weight and hatch)
0%[a]	−0.0953±0.0039[c]	−1.3033±0.0737[c]	0.978***
0%[b]	−0.0606±0.0090	−0.8696±0.1082	0.972***
30%	−0.0339±0.0032	−0.4597±0.0479	0.971***
60%	−0.0210±0.0012	−0.2760±0.0684	0.957***
75%	−0.0206±0.0010	−0.2465±0.0148	0.940***
90%	−0.0177±0.0008	−0.2245±0.0087	0.918***

[a] "Optimally" conditioned eggs.
[b] Suboptimally conditioned eggs.
[c] Standard error of the regression coefficients.
*** Probability less than .001.

Some eggs which failed to hatch on the first attempt hatched after further conditioning treatment. However they progressively lost this ability to be reconditioned. The point in time when the eggs did not respond to further conditioning coincided with the time when first-attempt hatching ceased.

DISCUSSION

Christophers (1960) lists the following weights for eggs of *A. aegypti*: 4 days old, 11.1 ± 0.2 μg; 15 days old, 10.4 μg; 25 days old, 10.4 μg; slightly shrunken eggs, 7.5 μg; shrunken, prismatic eggs, 5.9 μg; flattened, saucerlike eggs, 4.0 μg. The conditions to which the eggs were exposed were not given, nor were the ages of the last three kinds of eggs.

Gander (1951) recorded the change in linear measurements of eggs of *A. aegypti* from laying to the point when they would hatch, as did Rosay (1959) with eggs of *Aedes nigromaculis* (Ludlow) and *Culex tarsalis* Coquillett, and Downs (1951) with anopheline eggs. In all cases the greatest increase in measurements occurred during the first few hours. *A. aegypti* eggs reached their final size at about 15 hours; in *A. nigromaculis* eggs, little or no change was observed after 10 hours.

Results of the present study of weight change substantiate the findings of Gander (1951), Rosay (1959), and Downs (1951) on change in linear measurements in that the period of most rapid growth of the egg is during the first several hours.

The observations of Christophers (1960) cannot be critically compared with those in the present study because of lack of information on the condition under which his experiments were done; however, the weights of 4-day-old eggs were similar in the two studies. His relatively large standard error of the mean indicates either a considerable amount of variation in egg weights or a small sample size.

Pertaining to the resistance of eggs, Gander (1951) states that the varying resistance to drying of *A. aegypti* eggs is due to the varying thickness of the chorion. Rosay (1955), working with *A. nigromaculis* eggs, states that ". . . the middle, or dark chorion . . . apparently controls water loss in protecting the embryo from excessive drying." Telford (1957) in experiments with *Aedes dorsalis* (Meigen) eggs found that eggs 20 to 27 hours old showed a sharp increase in resistance to desiccation. He believes that the "vitelline membrane" (transparent cuticle) must be largely responsible for this resistance. Beckel (1958) observed two changes in permeability in the prediapause stage of the eggs of *Aedes hexodontus* Dyar. The first took place as the chorion changed from white to black. With the formation of the transparent cuticle a further change in permeability occurred. Since removing the chorion rendered the cuticle quite permeable he believes that ". . . the impermeability of the chorion and cuticle combination must result from the bonding between the two and this bonding must be disrupted by the dechorionation."

Observations on eggs of *A. aegypti* in the present study substantiate some of the observations of these authors. The flattening of the weight-gain curve in figure 1 corresponds with the time of formation of the transparent cuticle. Prior to the formation of

this layer the eggs readily collapsed in atmospheres of low humidity, this being especially true of the soft, flexible eggs just after laying. At about 1 hour the chorion darkened, became somewhat rigid, and the egg attained a certain amount of resistance to desiccation. At the time of formation of the transparent cuticle the eggs became most resistant to desiccation and their gain in weight virtually ceased.

As to the length of time *Aedes* eggs can remain viable, Bacot (1918) found that a very small percentage of *A. aegypti* eggs hatched at 15 months. Eggs, in this case, were stored on filter paper " . . . in a wax card jar kept in a cool room (ca. 9° C.) . . ." Shannon and Putnam (1934) state that about 5% of the eggs of *A. aegypti* which are ripened on moist filter paper, then dried and stored, will survive a year or more. No mention is made of storage conditions. Woodhill (1948) working with *A. aegypti* and *Aedes scutellaris* (Walker) found that the eggs of the former were much more resistant to desiccation than were those of the latter.

Under natural conditions in which temperatures varied from 5° to 32° C. and humidities varied from 19% to 100%, *A. aegypti* eggs remained viable for up to 87 days. In these experiments eggs were placed outdoors and protected from rain. Other experiments in which relative humidity was kept high (90% to 100%) and temperatures kept at −1° to 4° C. showed that both *A. aegypti* and *A. scutellaris* eggs remain viable up to 14 days, though the former gave a greater percentage hatch. Surtees (1958) found that *A. aegypti* eggs subjected to field conditions in southern Nigeria, commencing in October, remained hatchable for 24 weeks, but did not hatch after 28 weeks. Ingram (1954) working with *Aedes polynesiensis* Marks in the laboratory reported that, "mature eggs may be dried and remain viable for 30 days or longer. . . . Undried eggs continued to hatch for a period of 72 days." These eggs were dried in the laboratory where ". . . the relative humidity . . . was usually less than 75%." Mail (1934) found that some eggs of *Aedes campestris* Dyar and Knab, *A. dorsalis*, and *Aedes vexans* (Meigen) would still hatch after 20 months when flooded with water. In these experiments eggs were held in glass vials, some without moisture and some in pond water in a cold chamber at 0° to 10° C. Yates (1945) found that *Aedes sticticus* (Meigen)[7] eggs were more resistant to desiccation than were eggs of *A. vexans*. Some eggs of both species were viable after a year. In these experiments eggs were not separated from soil. Soil samples, containing eggs, were stored in a basement room that was ". . . dry and [had] a uniform temperature close to 70° F." Gjullin and Yates (1946) reported that some eggs of *A. vexans* and *A. sticticus* hatched after 5 and 7 years respectively. Soil samples, containing these eggs, were kept in the field and brought into the laboratory for testing.

[7] This species was known as *Aedes lateralis* (Meigen).

The varied results of these observers are, no doubt, due to the varied conditions under which the eggs were held during the course of experimentation. In most cases no attempt was made to control environmental conditions and in those tests conducted in the laboratory, control of the environment was not strict. It appears, however, that in general longevity increased with increased relative humidity (though some observations cast doubt on this); results of the present study clearly show this to be true.

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Aphytis lepidosaphes (Hymenoptera: Chalcidoidea), an Introduced Parasite of Purple Scale[1]

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ABSTRACT

The introduced chalcid parasite, *Aphytis lepidosaphes* Compere, became a major factor which contributed to the decreased importance of purple scale on citrus in the Lower Rio Grande Valley of Texas. A technique of evaluation of parasite activity was developed in a 42-month period. Parasite activity was determined through periodic recording of the number of scale with live, immature parasites, which proved to be a valid estimate of increasing and decreasing trend of parasite numbers. Greatest parasite activity occurred during periods of higher relative humidity. Percentage parasitism records appeared to have little value except that 30% or higher percentages were associated with smaller scale numbers.

Purple scale, *Lepidosaphes beckii* (Newman), was ranked the fourth most important pest of citrus in the Lower Rio Grande Valley area in 1950. The internal, purple scale parasite, *Aspidiotiphagus citrinus* (Craw.), had been of only incidental importance; so, an external parasite, *Aphytis lepidosaphes* Compere, was introduced in July 1952 to attempt to increase the biological control of purple scale.

A. lepidosaphes was introduced into California on December 1, 1948 from the Kwangtung Province of South China according to Smith and Flanders (1949, 1950). Flanders (1950) reported the parasite produced about two generations to one of the scale under laboratory conditions. Original description of the parasite was made by Compere (1955).

Adults were reared in the insectary at Riverside, California from field-collected purple scale parasitized by *A. lepidosaphes* and shipped via airmail to Weslaco. The first lot of parasites was released in a grove south and west of Weslaco on July 17, 1952, and a second lot (approximately 1,200) was released in the same grove on July 19. Ten parasites of the latter lot were released at Substation No. 15. Another lot was released in a grove 2 miles east of Mercedes on July 23. Dissemination of the parasite in this area has been principally through natural means. However, parasitized scales were carried to a grove at Bayview, Hoblitzelle Ranch north of Mercedes, and 2.3 miles north Bass Boulevard west of Harlingen during 1955 and early 1956. The evaluation period extended over a period of 42 months.

PROCEDURE

A technique was developed to evaluate the extent of parasitism by *A. lepidosaphes*. The percentage of live, parasitized, and dead scales was determined during the preliminary stages of the study. The "dead scale" figure considered scales which were dead from causes other than by parasites, such as predator action, old age, etc. A dead scale figure can be found from the tables by subtracting the live scale and parasitized scale percentages from 100. No confusion with other parasites was expected as *lepidosaphes* has been the only external parasite of third stage purple scale on citrus known in this area. Any scale with a parasite emergence hole or with live *lepidosaphes* was considered parasitized. However, it soon became evident that a percentage parasitized scale figure failed to reflect a valid increasing and decreasing trend of the parasite. Beginning in August 1956, eggs, larvae and pupae of the parasite were recorded thereafter, since these figures represented a more valid estimate of the degree of parasite activity.

Random samples of leaves were taken in approximately the same trees in the selected groves by collecting one infested leaf from each quadrant around the tree. Leaves were sampled from each quadrant because of the possibility of irregular scale or parasite populations such as reported for California red scale, *Aonidiella aurantii* (Maskell), by Ebeling (1950) and for various mites species by Dean (1959). Not more than 15 third-stage females were examined from each leaf under 18X magnification with a stereoscopic microscope until a composite sample of 500 scales was recorded.

Purple scale was sampled at monthly intervals until live-parasite forms were recorded in 36 records from each grove. Purple scale was abundant in each grove prior to the time of initial records. The relative scale density was indicated by the ease or difficulty of collecting samples for microscope examination.

Temperature and humidity data were taken from a recording hygrothermograph located at Substation No. 15, Weslaco. The distances to the various groves from Substation No. 15 were as follows: Hoblitzelle, 5 miles northeast; Melton, 0.2 miles south; Adams Gardens, 11.3 miles east northeast; and Tower, 10.7 miles northwest. Although the temperatures and humidity conditions may have varied somewhat in the outlying groves from those expressed in this paper, these differences were not considered of a sufficient magnitude to warrant possible changes in the general statements made regarding possible climatic effects on scale and parasite numbers.

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RESULTS AND DISCUSSION

The period of greatest increase of live scale generally occurred each year during the June-September period as shown in the table 1. The percentage of live scale usually decreased as the percentage of parasitized and/or dead scale increased. The June-September increase period was preceded by the following temperature conditions in number of hours per day above indicated temperatures: 1 above 90° F., 5 to 10 above 80° F., and 15 to 20 above 70° F. The summer live-scale increases occurred when the temperatures were 3 to 7 hours per day above 90° F. and more than 10 hours per day above 80° F. The higher peaks of purple scale populations generally were found during periods of greater number of hours per day below 50% relative humidity.

A greater number of scales with immature, live parasites was associated with the decreasing trend of live scale during the November-April period. The percentage of live scale generally decreased during this period. When 1.0% or less scale with live parasites occurred during the September-October period, the live-scale percentage increased, or decreased very little. This condition was especially true during the 1957-58 period. Generally, it appeared that live-parasite percentages (during November-April) of 2.5 to 5.0 or greater were necessary to depress live-scale percentages below the 30% level. A

Table 1.—Live purple scale and *Aphytis* parasitism records from grapefruit at various locations.

	Station			Hoblitzelle			Melton			Adams Gardens			Tower Road		
	A[a]	P[b]	L[c]	A	P	L	A	P	L	A	P	L	A	P	L
							1956								
March	5	8								30	0.0		5	28	
April	12	15		3	20		11	32		32	1.0		7	31	
May	15	21		5	31		17	36		58	7.0		8	37	
June	70	13		10	24		56	27		30	41		16	37	
July	75	17		77	8		33	58		47	36		55	24	
August	52	32	15.4	88	3		36	46	8.2	6	63	2.2	53	29	
September	41	40	3.4	76	5	2.2	16	53	4.8	13	62	2.2	60	20	5.6
October	21	37	.8	41	7	1.0	19	41	.8	3	63	.8	39	30	2.6
November	21	36	1.2	29	14	9.2	12	36	2.4	3	63	2.6	31	21	3.8
December	18	36	1.8	30	16	4.6	22	38	2.2	5	55	2.0	22	25	2.8
							1957								
January	20	23	4.8	17	26	7.2	24	34	6.2	3	53	1.4	21	23	5.8
February	11	31	2.6	12	29	3.2	24	32	6.2	3	57	.4	15	26	5.0
March	18	26	1.8	7	29	3.4	11	37	3.8	4	61	2.4	8	34	3.6
April	17	32	1.0	5	29	2.6	10	35	1.4	6	61	2.6	8	41	2.2
May	14	31	3.2	5	32	1.2	11	40	2.0	4	55	1.4	13	37	2.0
June	11	33	4.2	24	25	1.0	19	31	1.4	7	57	1.6	28	31	.6
July	11	47	2.6	49	26	2.6	42	31	2.0	8	65	2.2	49	20	1.6
August	40	33	.6	50	17	.2	43	34	.4	21	47	1.6	69	12	1.6
September	30	35	.6	57	13	.2	44	24	.0	21	50	1.6	64	12	2.0
October	37	25	.6	40	13	.4	34	33	.6	19	42	.4	66	8	.8
November	52	15	.4	26	16	2.6	24	21	.4	30	35	1.6	54	6	.2
December	35	19	3.4	25	13	1.4	34	23	.2	24	28	1.0	32	6	.2
							1958								
January	62	8	1.6	27	13	2.8	38	20	.0	35	18	.6	35	3	.4
February	50	14	6.0	20	18	3.8	36	19	.0	50	17	.8	22	6	.4
March	52	9	1.2	25	17	2.0	45	13	1.0	43	21	.2	32	6	1.6
April	37	6	2.2	21	18	4.4	31	13	2.8	35	19	1.6	22	10	3.8
May	25	25	8.6	24	20	.6	26	24	6.8	52	4	.6	7	11	2.6
June	31	24	6.6	54	12	2.0	25	39	8.6	54	19	9.2	23	11	.0
July	52	26	6.8	58	12	.8	37	34	1.8	63	12	4.0	50	7	.2
August	59	29	9.8	58	27	2.4	32	30	3.6	39	29	2.0	57	6	1.4
September	63	21	3.8	74	9	.4	22	29	.2	35	36	2.8	17	9	.4
October	13	30	2.2	57	6	1.4	19	32	1.4	26	28	1.4	14	14	1.2
November	12	30	3.4	50	6	1.4	35	17	.6	28	27	4.2	20	5	.4
December	20	22	3.2	40	10	4.4	27	22	4.0	33	38	7.6	22	7	3.2
							1959								
January	18	31	3.0	39	9	2.8	28	25	9.0	32	30	9.8	27	16	4.8
February	27	32	4.0	27	9	2.0	29	27	8.4	35	27	7.4	19	20	3.0
March	17	35	6.0	34	10	5.4	22	28	6.6	30	39	17.2	22	16	4.0
April	14	34	6.4	19	17	3.8	18	39	3.8	22	40	14.2	14	20	3.0
May	40	33	9.6	18	17	1.2	28	31	6.6	35	31	5.0	21	18	3.2
June	37	35	8.6	19	22	4.4	24	40	10.2	50	26	7.8	40	14	.6
July	44	33	9.4	48	17	3.4	33	42	10.8	26	28	2.4	45	15	6.0
August	60	29	18.6	58	18	2.4	33	45	16.2	24	34	9.2	33	42	20.8

[a] Percent live scale.
[b] Percent parasitism.
[c] Percent of scale with live *Aphytis* parasites.

smaller live-scale percentage followed the November-April periods which had greater percentages of scale with live parasites. Thus, *A. lepidosaphes* was found to be a regulator of live purple scale populations.

The percentage of scale with live parasites was generally above 2.5 from December 1958 through July 1959 at all locations. During this period of high parasite activity, the number of hours per day above 70% relative humidity was 19.7 and longer while 1.7 or less hours per day below 50% relative humidity occurred. Percentages of scale with live parasites were greater than 2.5 during the November 1956 through March 1957 period in the Hoblitzelle and Tower groves and during January 1957 in the Melton grove. Humidity conditions during the latter period varied from 9 to 15 hours per day above 70% and 3.4 to 5.8 hours per day below 50%. The live parasite population then decreased as did the live scale percentage before the normal summer increase of live scale. Percentages of scale with live parasites were also greater during the late spring and summer 1958 in the Adams Gardens, Melton and Station groves. Although increases of live parasite percentages were found under a range of humidity conditions, the continued high relative humidity conditions were more favorable for the maintenance of greater numbers of live *A. lepidosaphes*.

Generally, a small percentage of live parasites was found during the August-October period of 1957. It appeared the smaller parasite numbers would continue to prevail where the level of scale made it difficult to collect. This reduction in live-parasite percentages occurred frequently in the October samples if the reduction had not come during September. The reduction usually followed a period during which the temperature was about 7 hours per day above 90° F. However, the parasite maintained itself in very small scale populations, even in the Melton grove where it was difficult to collect enough scale to examine during the fall of 1957 and at other times.

The percentage of scale with live parasites usually increased during the summer months. Where this increase was not found, an increase in the percentage of live scale was usually evident. This is reflected in the general averages at the various locations as shown in the table 1. A greater live parasite population during this period showed a greater effect on the reduction of live scale in the period immediately following the summer scale increase period. However, the smaller live scale population was not evident until October in the Station grove during 1958.

Parasitism of 30% or greater was found to be associated with small population level of live scale. During the summer scale increase period the percentage of live scale might continue upward for a month or two but the downward trend of live scale was usually forthcoming when the parasitism continued above 30%. However, it should be pointed out that the percentage parasitism can be an accumulative figure and the greater live parasite percentages were not particularly associated with the greater parasitized-scale percentages. The percentage, parasitized-scale figure was not considered to be a valid estimation of parasite abundance. This was evident in the many records of greater percentage parasitism and very small percentage live parasites, as shown in table 1.

The percentage of dead scale, as a result of causes other than by parasites, followed a fairly uniform trend of increases and decreases. Smaller percentages of dead scale were usually found during the July-September period. This decrease occurred during the scale-increase period. Adults of the scale-predator *Chilocorus cacti* L. emerge during late August and September. The feeding on the scale by this beetle was probably one of the principal causes of increase of dead scale during the fall and winter seasons. Percentage of dead scale was also considered an accumulative estimate of population.

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Optic Organs of *Tribolium confusum* and *T. castaneum* and Their Usefulness in Age Determination (Coleoptera: Tenebrionidae)[1]

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ABSTRACT

Two pairs of stemmata are present in all larval instars and in the early pupa of *T. confusum* Jacquelin du Val and *T. castaneum* (Herbst). They degenerate in the pupa as the compound eyes begin to develop. Larval instars are separable by determining the distance between the two pairs of stemmata. The stemmata are located near the antennae from the first throughout the early part of the last larval instar. With each successive moult the distance between the stemmata increases. During the latter part of the last larval instar, however, the interstemmatal distance decreases as the stemmata migrate to a position near the vertex. The degree of stemmatal migration can be used as an index for determining the ages of last larval instars. The orientation of the stemmata in the latter part of the last larval instar also differs from that seen in other instars. The pupal age can be estimated by counting the number of rows and considering the degree of development of the ommatidia of the compound eyes.

The importance of the optic organs of *Tribolium* in taxonomic studies has been pointed out by Good (1936), who used features of the compound eyes of adults as criteria for separating species. Ho (1960) also used these structures to distinguish between the pupae of *T. confusum* Jacquelin du Val and *T. castaneum* (Herbst). The eyes in *Tribolium* larvae and pupae vary considerably in shape, size, color, location, orientation, interocular distance and facet arrangement. Thus, features of the optic organs of *Tribolium* can be used not only in taxonomy, but also in morphological studies and for age determination.

MATERIALS AND METHODS

All of the insects used in this study were reared in vials at 29° C. and 70% relative humidity. Each vial contained about 1 gram of a mixture of 95 parts whole wheat flour and 5 parts dried yeast. The larvae used for head capsule and interstemmatal distance determinations were reared from eggs. One egg, less than 1 day old, was placed in each of the vials; 45 larvae of *T. confusum* and 41 of *T. castaneum* were obtained in this manner. The growth of each larva was checked daily through the third instar; thereafter they were measured every other day. Ether was used to immobilize the larvae while measurements were made. Measurements were also made on nonanaesthetized individuals of the two species. In order to do this, about 50 eggs less than 1 day old were placed in each of five culture vials prepared as indicated above. Larval growth was checked daily by randomly selecting five or more from each vial. A hot petri dish was used to kill the larvae before measuring. In order to observe the migration of stemmata in the last larval instar, their position in the pupa, and development of compound eyes in the pupal stage, 20 last-instar larvae of each species were transferred to vials and reared separately. Head capsule and interstemmatal distance measurements as well as the stemmatal migration route were determined twice a day without using anesthesia. In the pupal stage development was checked once a day.

LARVAL STAGE

Marshall (1927) was of the opinion that each stemma of *T. confusum* is paired, with the two parts adjacent to each other. According to my observations, the stemmata of both *T. confusum* and *T. castaneum* are usually rod-shaped or dumbbell-shaped. When examined externally, seldom did they appear to be paired. An examination of sections, however, revealed that they were indeed paired, each pair being supplied by a biramous nerve which could be traced to the brain. The stemmata are dark brown or black in color, turning light brown and gradually disappearing from the hypodermis at pupation. The position of the stemmata and the interstemmatal distance varies with larval age. Interstemmatal distances are given in table 1 for *T. confusum* and *T. castaneum*, respectively, and it may be seen that these values are very similar for the two species.

The measurement of interstemmatal distance.— The larval instars of *Tribolium* cannot be accurately correlated with body length because of the range in each instar owing to the elasticity of the intersegmental membranes. Chapman (1918), Brindley (1930), and Good (1936) determined the larval instars of *T. confusum* by measuring the width of the head capsule. The present study demonstrates that the interstemmatal distance also can be used for this purpose. Table 1 indicates that either of these characteristics is dependable.

All the *Tribolium* used for these measurements belonged to a population synthesized from several strains. Overlapping values for head capsule and interstemmatal measurements were found in both species (table 1). These were caused by one or two undersized larvae in the cultures. The coefficient of variation indicates that interstemmatal distance is a more reliable criterion for instar determination than is head-capsule width. Table 1 indicates that head-capsule width and interstemmatal distance can be used simultaneously to determine the instar of the larvae, since in all the ages of the larvae except

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Table 1.—Head capsule widths and interstemmatal distance in the etherized larvae of *Tribolium* species.

Instar	Head capsule width (mm.)				Interstemmatal distance (mm.)				N
	$\bar{x}$	Range	σ	C.V.	$\bar{x}$	Range	σ	C.V.	
				Tribolium confusum					
1	0.160	0.150-.175	0.0082	5.1	0.122	0.112-.125	0.0054	4.4	38
2	.196	.175-.225	.0093	4.7	.148	.138-.150	.0047	3.2	42
3	.261	.225-.300	.0153	5.9	.198	.188-.225	.0079	4.0	45
4	.353	.300-.375	.0114	3.2	.267	.225-.275	.0114	4.3	44
5	.470	.375-.525	.0325	6.9	.364	.275-.400	.0209	5.7	44
6	.608	.500-.650	.0314	5.2	.477	.400-.525	.0260	5.4	43
7	.661	.612-.688	0.306	4.6	.530	.512-.550	.0125	2.4	7
				Tribolium castaneum					
1	0.156	0.150-.162	0.0063	4.0	0.117	0.100-.125	0.0063	5.4	38
2	.190	.175-.225	.0153	8.0	.145	.125-.175	.0101	7.0	41
3	.242	.200-.262	.0106	4.4	1.80	.150-.200	0.120	6.7	47
4	.326	.275-.350	.0221	6.8	.243	.225-.275	.0141	5.8	35
5	.449	.350-.488	.0285	6.3	.342	.288-.362	.0154	4.5	36
6	.590	.475-.638	.0345	5.8	.467	.400-.500	0.180	3.9	35
7	.662	.575-.712	.0395	6.0	.548	.488-.575	.0223	4.1	12

N = number of larvae measured.
$\bar{x}$ = average of measurements.
σ = standard deviation.
C.V. = coefficient of variation (in percent).

the latter part of the last instar these values increase constantly. Under the described rearing conditions the usual number of larval instars was six, but in some larvae seven instars were found in both species. In comparing the measurements obtained after etherization with those obtained after heat killing, the latter method yielded slightly larger and more accurate values than the former. No overlapping was found in the measurements of the interstemmatal distances after the heat killing. This may be due to the total inactivity of the larvae during measurement as compared to only partial inactivity after etherization. However, the heat-killing method only can be applied to the larvae to be discarded.

The stemmata are located laterally on the postfrontal region of the head near the antennae until the early part of the last instar. During the last larval stadium they migrate to a position near the vertex. That this change in position is due to mi-

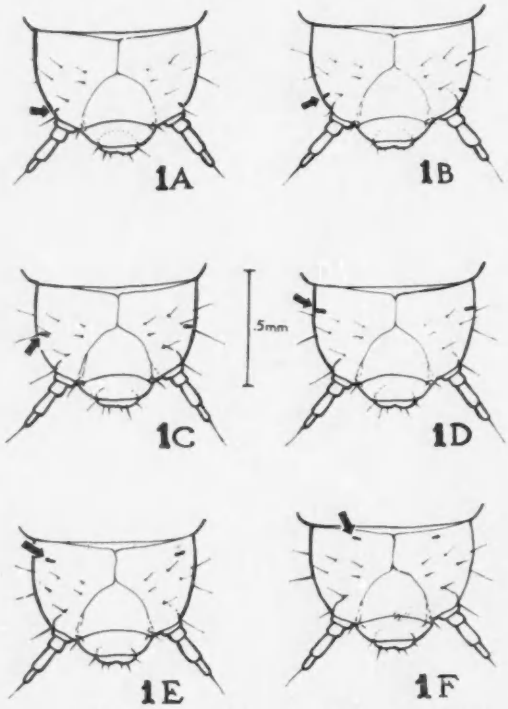

FIG. 1.—Ventral view of heads of last instar *Tribolium confusum* larvae, showing the migration route of the stemmata. A. One to two days old. B. Three days old. C. Four days old. D. Four to five days old. E. Five to six days old. F. Six to seven days old.

Table 2.—The route of stemmatal movement in the last larval instar of *Tribolium*.

Day and time measured	*T. castaneum*			*T. confusum*		
	Interstemmatal distance (mm.) mean	range	Fraction of total distance covered by stemmata	Interstemmatal distance (mm.) mean	range	Fraction of total distance covered by stemmata
1st A.M. through 3rd A.M.	0.50	0.50	1/8-1/4	0.50	0.50	1/8
3rd P.M.	.50	.50	1/4-3/8	.50	.50	1/8-1/4
4th A.M.	.48	.40-.50	3/8-1/2	.50	.50	1/4-3/8
4th P.M.	.48	.40-.50	1/2-5/8	.49	.48-.50	3/8-1/2
5th A.M.	.41	.35-.45	5/8-3/4	.47	.38-.50	1/2-5/8
5th P.M.	.40	.35-.45	3/4-7/8	.43	.38-.48	5/8-3/4
6th A.M.	.37	.35-.40	7/8-1	.40	.38-.48	3/4-7/8
6th P.M.	.36	.32-.40	1	.39	.35-.42	7/8-1
7th A.M.	—	—	—	.39	.35-.42	1

gration rather than absorption and reformation is indicated by the fact that the migration route can be traced from point to point, as shown in figure 1 for *T. confusum*. This figure also applies to *T. castaneum*. In general, the duration of the last larval instar in the two species of *Tribolium* is from 6 to 7 days. During this period of stemmatal migration, on the average roughly a quarter of the complete distance is traversed each day. In the earlier and later stages of migration, the change is

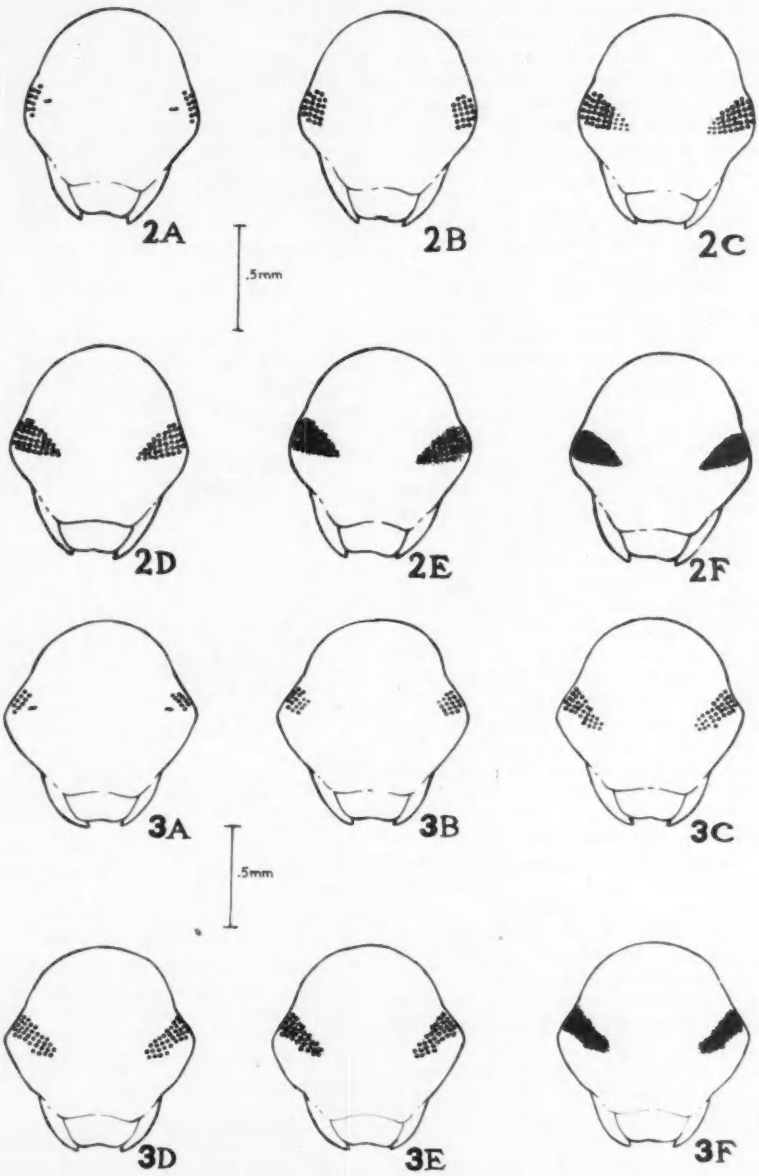

FIG. 2 and 3.—Ventral view of heads of *T. castaneum* pupae (FIG. 2) and *T. confusum* pupae (FIG. 3), showing the arrangement of the facets of the compound eyes. Hairs, antennae and some mouth parts are omitted. A. One day old (note stemmata). B. Two days old. C. Three days old. D. Four days old. E. Five days old. F. Six days old.

more gradual than in the middle stages. From figure 1 and table 2, it is evident that the interstemmatal distance shows almost no change during the first days of migration. When the stemmata have passed through three-quarters of their route and are close to the posterior margin of the head, they move medially. This final position in the mature larva is nearly halfway between the ecdysial line and the side of the head. At this time the eye color has changed from black to brown and the eye shape from rodlike to circular or irregular. In this stage the larvae seldom feed, they move slowly, their bodies shrink to some degree and they are ready to pupate. This prepupal period lasts only about 1 or 2 days at 29° C. and 70% relative humidity.

The orientation of the stemmata in the larvae also varies with age. When located near the antennae, the rodlike or dumbbell-shaped stemmata have their long axes oblique to the longitudinal axis of the head and the upper end is always directed inward (fig. 1). When the stemmata have migrated through half of their route, they become transversely oriented.

PUPAL STAGE

Stemmata (figs. 2A and 3A).—The stemmata can be seen only during the first two days of the pupal stage. They usually disappear as soon as the facets of the compound eyes have partially developed. In certain individuals of *T. confusum* and *T. castaneum* the stemmata may not be clearly visible at any time during the pupal stage. Marshall (1928) found from an examination of sections that soon after pupation begins each stemma starts to withdraw from the surface of the head until it finally reaches the apex of the optic lobe. He believed that this movement of stemmata in insects is due to the "contraction" of the ocellar (stemmatal) nerves. The wandering of the stemmata also has been described in a number of insects by Günther (1911) and Corneli (1924). The stemmata in *Tribolium* do not invariably disappear from the hypodermis at the same age, nor do they always disappear on the two sides at the same time. As a result, some pupae during the first 2 days are equipped with a pair of apparent stemmata, others have no visible stemmata, and still others have the stemmata on one side showing more clearly than on the other.

Compound Eyes.—The first visible sign of the development of the compound eye in the pupal stage of *Tribolium* appears on the first day. The facets closest to the gena develop earlier than the others. In 1-day-old pupae (figs. 2A and 3A) two rows of brown facets are located directly above each dark gray or black stemma, in an arrangement much like a human eye and eyebrow. In 2-day-old pupae (figs. 2B and 3B), four or five rows of facets on each side may be seen and the stemmata are faintly visible as mere shadows. In 4-day-old pupae (fig. 2D, 3D, 4A and 4B), all the facets may be well developed, but in 3-day-old pupae (fig. 2C and 3C), the facets near the medium apex are smaller and lighter in color than the others. By the fifth day, (figs. 2E and 3E), the background of the eye area has turned to a light brown color. The eye area becomes entirely dark brown or black by the sixth or seventh day (figs. 2F and 3F).

The shape of the compound eyes of *Tribolium* pupae appears different in ventral and lateral views. From the ventral side only the ventral lobe of the eye is visible, but both ventral and dorsal lobes of the compound eyes can be seen laterally. Seen ventrally, the compound eye of *T. confusum* pupae appears as a strip while in *T. castaneum* it appears nearly triangular. Seen laterally, the compound eyes in *T. confusum* are nearly, but not completely, divided by the genae, while those in *T. castaneum* are only slightly emarginated (figs. 5A and 5B).

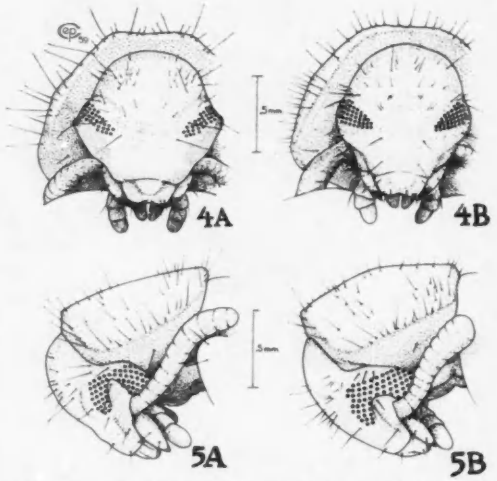

FIG. 4.—Ventral view of heads of four-day-old pupae, showing the arrangement of the facets of the compound eyes, the shape of the genae, and the interocular distance. A. *T. confusum*. B. *T. castaneum*.

FIG. 5.—Lateral view of heads of four-day-old pupae, showing the shape of the compound eyes. A. *T. confusum*. B. *T. castaneum*.

ADULT STAGE

The compound eyes of *Tribolium* adults (figs. 6A and 6B) are very similar to those of the late-stage pupae. However, because the head of the adult is less deeply set into the prothorax, the dorsal lobe of the compound eye (not figured) can be seen clearly in dorsal view.

OPTIC ORGANS IN *Tribolium* MUTANTS

The compound eyes in *Tribolium* normally appear black when the adult emerges. A mutation, "pearl," has been reported in both *T. castaneum* (Park 1937, Sokoloff 1959) and *T. confusum* (Graham 1957). In the mutants only the peripheral ring of ommatidia

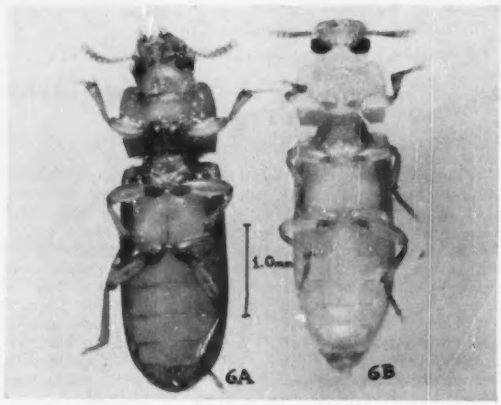

Fig. 6.—Ventral view of *Tribolium* adults, showing the shape of the compound eyes and the interocular distance. A. *T. confusum*. B. *T. castaneum* (freshly emerged).

appears black, the central facets are devoid of pigment; this is due to the ocular diaphragm which is pigmented black. In normal larvae the stemmata are black or brown; in "pearl" larvae the stemmata are not visible. In "pearl" pupae the facets of the compound eye also lack pigment, except that in the late pupa, with the development of the ocular diaphragm, the peripheral facets may appear brown or black. These features have been observed in other tenebrionid flour beetles, *Latheticus oryzae* Waterh. and *T. castaneum* by Sokoloff (1959a), and *Gnathocerus cornutus* (Fab.) by the author. It is evident that, because of a lack of pigment through most of the juvenile stages, the method of age determination described in the preceding paragraphs cannot be applied to these mutants.

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. I. Michael Lerner of the Department of Genetics, University of California, for his interest during the course of this study, to Dr. Rudolph L. Pipa of the Department of Entomology and Parasitology, University of California, and to Dr. Alexander Sokoloff of the Department of Genetics, University of California, for reading and criticizing a first draft of this paper. It is also a pleasure to acknowledge the assistance of Mr. Peter Dawson, who made most of the measurements and calculations on the larval stage, and Mr. Paul Catts, for drawing the excellent illustrations. Permission has been obtained from the editor of the Annals of the Entomological Society of America to reproduce figure 4.

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SCIENTIFIC NOTES

Guanine Excretion by the Two-Spotted Spider Mite (*Tetranychus telarius* (L.))[1]

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Two purines have been shown to be nitrogenous end products in arthropods, namely uric acid in insects and guanine in spiders (Prosser 1950).

Blauvelt (1945) found spherical concretions in the hindgut of the two-spotted spider mite which he classed as urates. As no malpighian tubes were present, the glandular cells in the anterior lateral walls of the hindgut (plate 2, Blauvelt 1945) were described as a specialized nephritic organ for the elimination of urates. Gasser (1951), however, on the basis of solubility tests, described not only the concretions in the hindgut but also part of the black fecal pellets as containing guanine. These spherical concretions accumulate in the hindgut and are passed with the fecal pellets.

Examination of these concretions in the hindgut with the polarizing microscope in the present study showed them to be strongly birefringent, indicating a crystalline structure. However, neither Li_2CO_3 extract nor whole crushed mites gave a murexide test typical for uric acid. The crystalline structure was found to be enclosed in a stroma which stained with both sudan III and amaranth red, indicating that the stroma may contain a lipoprotein.

Adult female mites were extracted for purines in the following manner. Mites were homogenized in 1N NaOH, centrifuged and the supernatant saved, adjusted to pH 3 with H_2SO_4, and recentrifuged. The supernatant was heated on a water bath for 5 minutes, then centrifuged, and the precipitate discarded. Several milliliters of 0.1N $AgNO_3$ were added until no more white precipi-

[1] This investigation was supported by a grant (RG6109) from the National Institutes of Health, Public Health Service. Accepted for publication May 1, 1961.

tate formed, and the vessel was held overnight in the refrigerator. A light, flocculent precipitate was decanted and the white crystalline residue was washed three times with distilled water. The purines were discharged from their silver salts with 1N HCl, the mixture centrifuged, and the supernatant used to test for purines.

The supernatant along with guanine in 1N HCl was examined in the U.V. region from 230 to 320 mμ. Both spectra were in agreement, with maxima at 250 mμ.

The extract, guanine plus extract, and guanine were run on Whatman No. 1 paper using the following solvent systems: tert-butanol: 0.6M NH_4OH 6:1, and tert-butanol, formic acid, water 77:11-12. The spots were visualized with a 0.25% mercuric acetate ethanol spray followed by a 0.5% diphenyl carbazone ethanol spray (Block et al. 1958).

Only one spot was found, with an Rf of 0.24 in the ammonia system and Rf of 0.70 in the formic acid system. The mixed spot could not be resolved into more than one spot.

When silk, with accumulated fecal material, was extracted by the same procedure only guanine could be identified in the extract, thus guanine is the only purine excreted by the mite.

With the above results indicating the presence of only guanine, Gomori's (1952) methylamine silver stain was used to localize the guanine in the mite. The stained section in figure 1 showed the staining reaction localized in the concretions of the hindgut. As no staining reaction was found in the fat body, perhaps this organ does not function in the synthesis of purines as does the fat body of insects (McEnroe and Forgash 1958).

Although mites pass large volumes of water when feeding, and thus would not seem to require such a highly insoluble waste product as guanine, they have retained this ability. This excretion product may, however, serve the non-feeding diapause form and molting forms which are subject to water stress.

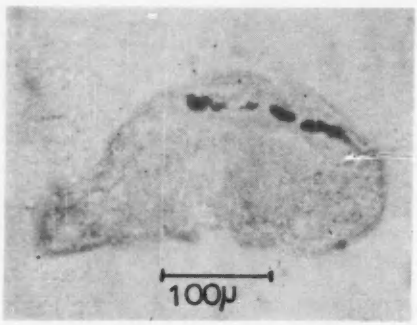

FIG. 1.—Mid-sagittal section of mite showing stained concretions in hindgut.

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A Simple Apparatus for Measuring Density of Insect Population[1,2]

RICHARD G. WIEGERT[3]

Measuring population density is often difficult in field studies of insects. Direct counts of individuals per unit area are possible only with slow-moving species. The sweep net, in wide use for sampling the more active forms, gives only an estimate of relative numbers. A conversion factor, derived from some direct method of sampling, must be used for expressing sweep data in terms of absolute numbers (Beall 1935; Gray and Treloar 1933; and Romney 1945). For sampling the entire above-ground insect fauna the suction-type apparatus of Dietrick et al. (1959) possesses the most general applicability for different vegetational types and species. The suction apparatus, however, is expensive and an extensive laboratory setup is needed for separation of the material collected. If an investigator is interested only in one or a few species of the insect fauna, it is sometimes possible to utilize a faster and less expensive sampling procedure designed specifically for a restricted group of species, such as that described in this note.

The apparatus was built expressly for sampling populations of adult meadow spittlebugs, *Philaenus spumarius* (L.), on an old field in southeastern Michigan. As the method subsequently proved applicable to other species which jump or fly in a manner similar to *P. spumarius*, this equipment should prove useful to biologists studying other insect populations.

An adult meadow spittlebug is in general sedentary and feeds on the juices of herbaceous plants. One can approach to within a few feet of these insects without putting them to flight, but as soon as the food plant is touched the bug quickly jumps from the plant and flies a short distance. These facts, plus the sparseness of the vegetation on this particular field, led me to develop the sampling apparatus shown in figure 1. The frame was made of lightweight strap iron, riveted and welded at the points of contact and covered with nylon screen. The upper ring was made somewhat smaller than the lower ring so that the metal cone would not be unwieldy. A Mason jar lid was soldered to the tip of the metal cone so that the jar could be removed. Cross-sectional details of the attachment of the nylon screen at the cone and bottom ring are shown at the right of the figure. The bottom ring encloses an area of 0.2 square meter, which was found to be about the largest size permitting efficient manipulation of the trap.

In collecting, the investigator approaches to within two paces of the area to be sampled. Holding the cone uppermost, he steps quickly forward and places the trap over the vegetation, forcing the lower ring down firmly against the ground. Next a flat piece of Masonite is placed on the ground next to the trap and the trap is worked onto the Masonite, using a twisting motion and keeping the lower edge of the ring as tight against the ground as possible. The enclosed vegetation is at first shaken and later flattened down as the trap is moved over it. As a result the insects jump onto the screen covering the sides of the cage. When the trap is completely on the Masonite and all vegetation has been pulled from beneath the lower ring, the entire apparatus is inverted, keeping the Masonite in place. By tapping the screened sides of the trap, all the insects are knocked down into

[1] Sponsored for publication by Thomas E. Moore, and accepted May 6, 1961.

[2] Support for the construction of the apparatus was provided by National Science Foundation grant G-13521 to the University of Michigan, under the supervision of Francis C. Evans. Thanks are due to him and to Thomas E. Moore for criticism of the manuscript.

[3] Department of Zoology, University of Michigan, Ann Arbor.

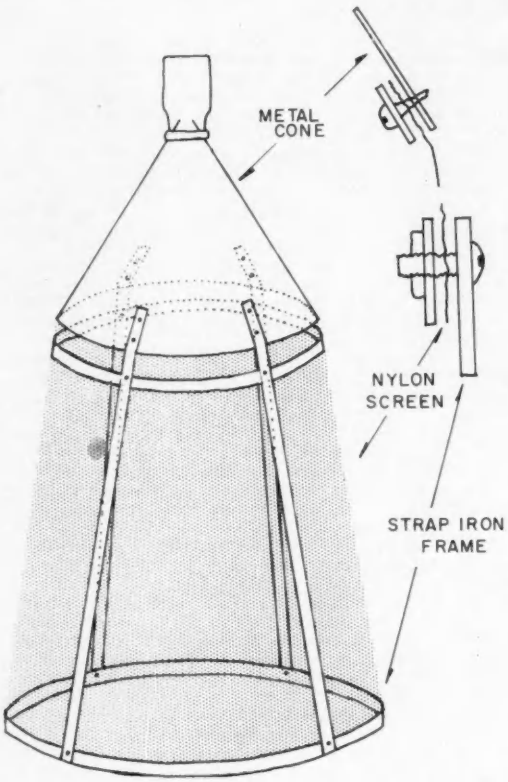

FIG. 1.—The trap used for sampling insect density showing details of the attachment of the screen.

the cone and shaken into the jar. A few drops of carbon tetrachloride added to the jar quickly kill the sample.

During the summers of 1959 and 1960 sets of 50 samples each were taken every 3 or 4 days. Standard errors of the mean were usually 10%-15%. The population size ranged from 0.39 to 26.0 spittlebugs per square meter. Sometimes a few meadow spittlebugs did not jump onto the screen, but remained trapped in the flattened vegetation after the cage had passed over it. These insects were readily captured by hand and included in the sample. The number of insects missed in this manner was in all cases less than 10% and usually less than 1% of the total number captured in any set of 50 samples. The time required to collect a single sample was about 3 minutes, including time spent walking between stations and counting the insects.

The apparatus also was used for sampling those species of grasshopper which permit close approach without flying, but which quickly leave the vegetation when it is disturbed. Forms which are burrowers or runners, such as crickets, often escaped capture, either by crawling into holes or finding an opening under the trap as it was moved onto the board. Insects capable of sustained flight, such as flies and wasps, were difficult to force down into the jar from the inverted trap. The method was of limited usefulness in dense vegetation because numbers of the insects failed to disentangle themselves from the plants and could not be readily found and collected for the sample.

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MOSQUITO CATALOG SUPPLEMENT

A Synoptic Catalog of the Mosquitoes of the World, Supplement I (Diptera: Culicidae) by Alan Stone brings the Catalog up to date with corrections and additions. This Supplement appeared in the *Proceedings of the Entomological Society of Washington* 63(1):29-52, March 1961. Price of the Catalog is $7.25, of the Supplement with covers $1.00. When the two are ordered together the price is $8.00 Prices to members when personally purchased are $6.50, 75 cents, and $7.00, respectively. Order from:

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BOOK REVIEWS

FRANCIS WALKER'S APHIDS. By JOHN P. DONCASTER. 1961. 165 pages,, 11 text figures, 8 plates. British Museum (Natural History). Price: Three pounds, from British Museum (Natural History), Cromwell Road, London, S. W. 7, England.

This is a concise, authoritative treatment of the aphids named and described by Francis Walker. It is a most useful volume, for many of Walker's aphid species are widely distributed and of recognized economic importance. The aims and accomplishments of the study are well described in the preface by W. E. China, who stated, ". . . From an examination of this [Walker's] material Mr. J. P. Doncaster has found it possible firstly to establish beyond reasonable doubt those of Walker's species which have recognizable types, and secondly by interpreting the information contained in the descriptions, host plant records and other available data, to identify at least some of those species which have no types. The purpose of the present volume is to make known the results of Doncaster's studies and thus to dispel at least some of the confusion and uncertainty which for more than a century have obscured the identities of so many of Walker's aphid species."

The volume contains an interesting, enlightening account of Walker's aphid studies, of his slide collections, and of his dry, pinned insects which have now been mounted on slides. There is a list of valid specific names and 47 of those proposed by Walker fall in this category. Following this is a list of 80 names which are synonyms and a list of 44 Walkerian nomina dubia.

The greater part of the volume is devoted to treatment of the individual species and names. The specific names are arranged alphabetically, and each heading contains the original name followed by the combination currently accepted by Doncaster or the names with which the original one is synonymized, or indication of its status as a nomen dubium or a manuscript name. Under the headings there are pertinent citations, Walker's complete description and notes, indication of type designations and relevant observations and comments by Doncaster, and, for some species, a synthesis of published comments by other authors. There is also a redescription by Doncaster of the holotype or lectotype of each valid Walkerian species. Keys are not provided.

The text figures are line drawings of portions of the body of various species, and the plates contain photographs of small or large areas of the body or of entire insects. The illustrations aid in the recognition of the species depicted.

A list of non-Walkerian aphid species in the Walker Collections and their currently accepted identity is included. It is noted that several of Kaltenbach's species were represented by specimens from Prussia and were probably given to Walker by Kaltenbach himself. This list contains synonymical notes and host associations of some species. The volume concludes with a bibliography and an index.

This is the second volume published by the Trustees of the British Museum (Natural History) on Francis Walker's insects, and the first devoted to a group of the Hemiptera. The first volume was devoted to the Trichoptera.

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PROBLEMS IN THE ZOOGEOGRAPHY OF PACIFIC AND ANTARCTIC INSECTS. By J. LINSLEY GRESSITT. Pacific Insects Monograph 2. Ent. Dept., B. P. Bishop Museum, Honolulu, Hawaii, pp. 1-94. 1961. With appendices by T. C. Maa, S. Nakata, I. M. Mackerras, and L. W. Quate. Price $2.50 bound, $2.00 unbound.

The title of this paper is an understatement. Although problems are posed for future workers, Gressitt has really given an up-to-date summary of the zoogeography of Pacific insects. He has further documented the thesis advanced by Mayr in 1941 that faunistically the Pacific islands pertain not to the Australian Region as originally proposed by Wallace, but to the Oriental Region. Evidence on this point is now so overwhelming that the traditional textbook maps should probably be changed.

Under "Dispersal" Gressitt has summarized the results of his highly original trapping project. By ingenious nets on planes and ships over the Pacific and in Antarctic waters over 25 cubic kilometers of air have been screened. The results confirm the hypothesis that insects and mites could have been carried over long distances in the past, since even wingless forms have been trapped at high elevations up to 3000 kilometers from the nearest mainland source.

Gressitt finds that Wallace's line "is not as distinct a boundary for insects as it is for higher vertebrates, particularly mammals." He discussed the faunal characteristics of islands on both sides of the "Line" and also continental and oceanic islands in the Pacific and Antarctic. Maps are mostly based on his own researches in the Cerambycidae and Chrysomelidae.

The most pressing problems for the future are listed as follows: 1) Sources and histories of the faunas of isolated oceanic islands; 2) Nature and history of dispersal; 3) Nature of "southern continent" relationships, and geological history of these areas; 4) Relationships of the Oriental and Australian regions.

The number of appendices might have been expanded indefinitely, since detailed analyses of distributional patterns can best be done by specialists. T. C. Maa has given a summary of Machaerotid (Cercopidae) distribution, Mackerras has dealt with Western Pacific Tabanidae, Miss Nakata discusses distributional patterns in Phasmatodea and Quate ends the monograph with a paper on "Zoogeography of Pacific Psychodidae."

This is a valuable addition to the newly established monograph series on Pacific insects. The format is pleasing and the illustrations, including black and white halftones and colored plates, are excellent. The book will prove to be indispensable to all students of Pacific island zoogeography.

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Berkeley

BRITISH FLIES, VOL. VI. EMPIDIDAE (PART 2). By J. E. COLLIN, 1961. pp. 223-551, text figs. 74-205. Cambridge University Press, American Branch, 32 E. 57th St., New York 22, N. Y. $6.00.

This is the second of three separately published but continuously paged parts of a revision of the British empidid flies (see Foote, 1961, Ann. Ent. Soc. America 54(5): 701). Part II contains a complete discussion of the subfamily Hybotinae, and a partial treatment of the Empidinae; the latter will be completed in a projected third part by a discussion of the empidine genus *Hilara*.

A somewhat disturbing obstacle to the efficient use of this volume is the lack of uniformity in the use of the titles *genus* and *subgenus*. For instance, the key on p. 444 leads one to the subgenera of *Empis* sens. lat., yet in the following discussion, species names are combined with subgeneric names as binomials. Furthermore, the running heads refer to the genus and species on a particular page by number rather than by name. However, as in Part I of the series, Mr. Collin's taxonomy and illustrations are beyond any reproach. Part 2 uniformly continues the high standards in these areas of treatment of the subject.

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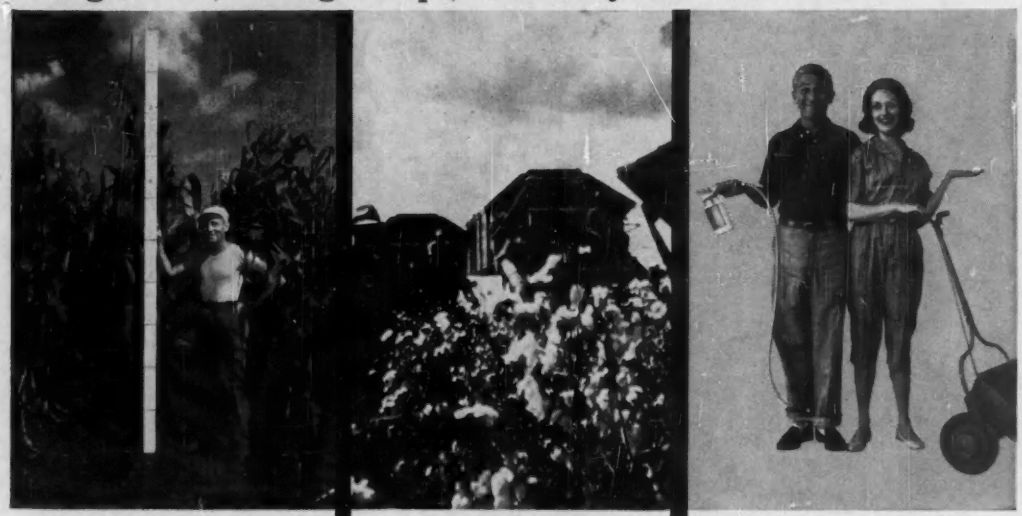

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